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(54) Title: NON-TRANSGENIC HERBICIDE RESISTANT PLANTS

(57) Abstract: The present invention relates to the production of a non-transgenic plant resistant or tolerant to a herbicide of the phosphonomethylglycine family, e.g., glyphosate. The present invention also relates to the use of a recombinagenic Oligonucleobase to make a desired mutation in the chromosomal or episomal sequences of a plant in the gene encoding for 5-enol pyruvylshikimate-3-phosphate synthase (EPSPS). The mutated protein, which substantially maintains the catalytic activity of the wild-type protein, allows for increased resistance or tolerance of the plant to a herbicide of the phosphonomethylglycine family, and allows for the substantially normal growth or development of the plant, its organs, tissues or cells as compared to the wild-type plant irrespective of the presence or absence of the herbicide. The present invention also relates to a non-transgenic plant cell in which the EPSPS gene has been mutated, a non-transgenic plant regenerated therefrom, as well as a plant resulting from a cross using a regenerated non-transgenic plant having a mutated EPSPS gene.

WO 01/24615 A1

NON-TRANSGENIC HERBICIDE RESISTANT PLANTS

The present application claims priority to U.S. Provisional Application No. 60/158,027, filed on October 7, 1999 and to U.S. Provisional Application No. 60/173,564, filed December 30, 1999, the disclosures of each of which are incorporated by reference herein in their entirety.

1. FIELD OF THE INVENTION

The present invention relates to the production of a non-transgenic plant resistant or tolerant to a herbicide of the phosphonomethylglycine family, *e.g.*, glyphosate. The present invention also relates to the use of a recombinagenic oligonucleobase to make a desired mutation in the chromosomal or episomal sequences of a plant in the gene encoding for 5-enol pyruvylshikimate-3-phosphate synthase (EPSPS). The mutated protein, which substantially maintains the catalytic activity of the wild-type protein, allows for increased resistance or tolerance of the plant to a herbicide of the phosphonomethylglycine family, and allows for the substantially normal growth or development of the plant, its organs, tissues or cells as compared to the wild-type plant irrespective of the presence or absence of the herbicide. The present invention also relates to a non-transgenic plant cell in which the EPSPS gene has been mutated, a non-transgenic plant regenerated therefrom, as well as a plant resulting from a cross using a regenerated non-transgenic plant having a mutated EPSPS gene.

2. BACKGROUND TO THE INVENTION

2.1 PHOSPHONOMETHYLGLYCINE HERBICIDES

Herbicide-tolerant plants may reduce the need for tillage to control weeds thereby effectively reducing soil erosion. One herbicide which is the subject of much investigation in this regard is N-phosphonomethylglycine, commonly referred to as glyphosate. Glyphosate inhibits the shikimic acid pathway which leads to the biosynthesis of aromatic compounds including amino acids, hormones and vitamins. Specifically, glyphosate curbs the conversion of phosphoenolpyruvic acid (PEP) and 3-phosphoshikimic acid to 5-enolpyruvyl-3-phosphoshikimic acid by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (hereinafter referred to as EPSP synthase or EPSPS). For purposes of the present invention, the term "glyphosate" includes any herbicidally effective form of N-phosphonomethylglycine (including any salt thereof), other forms which result in the production of the glyphosate anion in plants and any other herbicides of the phosphonomethlyglycine family.

Tolerance of plants to glyphosate can be increased by introducing a mutant EPSPS gene having an alteration in the EPSPS amino acid coding sequence into the genome of the plant. Examples of some of the mutations in the EPSPS gene for inducing glyphosate tolerance are described in the following patents: U.S. Patent No. 5,310,667; U.S. Patent No. 5,866,775; U.S. Patent No. 5,312,910; U.S. Patent No. 5,145,783. These proposed mutations typically have a higher K_i for glyphosate than the wild-type EPSPS enzyme which confers the glyphosate-tolerant phenotype, but these variants are also characterized by a high K_m for PEP which makes the enzyme kinetically less efficient (Kishore et al., 1988, Ann. Rev. Biochem. 57:627-663; Schulz et al., 1984, Arch. Microbiol. 137: 121-123; Sost et al., 1984, FEBS Lett. 173: 238-241; Kishore et al., 1986, Fed. Proc. 45: 1506; Sost and Amrhein, 1990, Arch. Biochem. Biophys. 282: 433-436). Many mutations of the EPSPS gene are chosen so as to produce an EPSPS enzyme that is resistant to herbicides, but unfortunately, the EPSPS enzyme produced by the mutated EPSPS gene has a significantly lower enzymatic activity than the wild-type EPSPS. For example, the apparent K_m for PEP and the apparent K_i for glyphosate for the wild-type EPSPS from *E. coli* are 10 μM and 0.5 μM, while for a glyphosate-tolerant isolate having a single amino acid substitution of alanine for glycine at position 96, these values are 220 μM and 4.0 mM, respectively. A number of glyphosate-tolerant EPSPS genes have been constructed by mutagenesis. Again, the glyphosate-tolerant EPSPS had lower catalytic efficiency (V_{max} /K_m), as shown by an increase in the K_m for PEP, and a slight reduction of the V_{max} of the wild-type plant enzyme (Kishore et al., 1988, Ann. Rev. Biochem. 57:627-663).

Since the kinetic constants of the variant enzymes are impaired with respect to PEP, it has been proposed that high levels of overproduction of the variant enzyme, 40-80 fold, would be required to maintain normal catalytic activity in plants in the presence of glyphosate (Kishore et al., 1988, Ann. Rev. Biochem. 57:627-663). It has been shown that glyphosate-tolerant plants can be produced by inserting into the genome of the plant the capacity to produce a higher level of EPSP synthase in the chloroplast of the cell (Shah et al., 1986, Science 233, 478-481), which enzyme is preferably glyphosate-tolerant (Kishore et al.,1988, Ann. Rev. Biochem. 57:627-663).

The introduction of the exogenous mutant EPSPS genes into plant is well documented. For example, according to U.S. Patent No. 4,545,060, to increase a plant's resistance to glyphosate, a gene coding for an EPSPS variant having at least one mutation that renders the enzyme more resistant to its competitive inhibitor, *i.e.*, glyphosate, is introduced into the plant genome. However, many complications and problems are associated with these examples. Many such mutations result in low expression of the mutated EPSPS gene product or result in an EPSPS gene product with significantly lower

enzymatic activity as compared to wild type. The low expression or low enzymatic activity of the mutated enzyme results in abnormally low levels of growth and development of the plant.

While such variants in the EPSP synthases have proved useful in obtaining transgenic plants tolerant to glyphosate, it would be increasingly beneficial to obtain a variant EPSPS gene product that is highly glyphosate-tolerant but still kinetically efficient, such that improved tolerance can be obtained with a wild-type expression level.

2.2 RECOMBINAGENIC OLIGONUCLEOBASES

Recombinagenic oligonucleobases and their use to effect genetic changes in eukaryotic cells are described in United States patent No. 5,565,350 to Kmiec (Kmiec I). Kmiec I teaches a method for introducing specific genetic alterations into a target gene. Kmiec I discloses, *inter alia*, recombinagenic oligonucleobases having two strands, in which a first strand contains two segments of at least 8 RNA-like nucleotides that are separated by a third segment of from 4 to about 50 DNA-like nucleotides, termed an "interposed DNA segment." The nucleotides of the first strand are base paired to DNA-like nucleotides of a second strand. The first and second strands are additionally linked by a segment of single stranded nucleotides so that the first and second strands are parts of a single oligonucleotide chain. Kmiec I further teaches a method for introducing specific genetic alterations into a target gene. According to Kmiec I, the sequences of the RNA segments are selected to be homologous, *i.e.*, identical, to the sequence of a first and a second fragment of the target gene. The sequence of the interposed DNA segment is homologous with the sequence of the target gene between the first and second fragment except for a region of difference, termed the "heterologous region." The heterologous region can effect an insertion or deletion, or can contain one or more bases that are mismatched with the sequence of target gene so as to effect a substitution. According to Kmiec I, the sequence of the target gene is altered as directed by the heterologous region, such that the target gene becomes homologous with the sequence of the recombinagenic oligonucleobase. Kmiec I specifically teaches that ribose and 2'-O-methylribose, *i.e.*, 2'-methoxyribose, containing nucleotides can be used in recombinagenic oligonucleobases and that naturally-occurring deoxyribose-containing nucleotides can be used as DNA-like nucleotides.

U.S. Patent No. 5,731,181 to Kmiec (Kmiec II) specifically disclose the use of recombinagenic oligonucleobases to effect genetic changes in plant cells and discloses further examples of analogs and derivatives of RNA-like and DNA-like nucleotides that can be used to effect genetic changes in specific target genes. Other patents discussing the use

of recombinagenic oligonucleobases include: U.S. Patent Nos. 5,756,325; 5,871,984; 5,760,012; 5,888,983; 5,795,972; 5, 780,296; 5,945,339; 6,004,804; and 6,010,907 and in International Patent No. PCT/US00/23457; and in International Patent Publication Nos. WO 98/49350; WO 99/07865; WO 99/58723; WO 99/58702; and WO 99/40789. Recombinagenic oligonucleobases include mixed duplex oligonucleotides, non-nucleotide containing molecules taught in Kmiec II and other molecules taught in the above-noted patents and patent publications.

Citation or identification of any reference in Section 2, or any section of this application shall not be construed as an admission that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention is directed to a non-transgenic plant or plant cell having one or more mutations in the EPSPS gene, which plant has increased resistance or tolerance to a member of the phosphonomethylglycine family and which plant exhibits substantially normal growth or development of the plant, its organs, tissues or cells, as compared to the corresponding wild-type plant or cell. The present invention is also directed to a non-transgenic plant having a mutation in the EPSPS gene, which plant is resistant to or has an increased tolerance to a member of the phosphonomethylglycine family, *e.g.*, glyphosate, wherein the mutated EPSPS protein has substantially the same catalytic activity as compared to the wild-type EPSPS protein.

The present invention is also directed to a method for producing a non-transgenic plant having a mutated EPSPS gene that substantially maintains the catalytic activity of the wild-type protein irrespective of the presence or absence of a herbicide of the phosphonomethylglycine family. The method comprises introducing into a plant cell a recombinagenic oligonucleobase with a targeted mutation in the EPSPS gene and identifying a cell, seed, or plant having a mutated EPSPS gene.

Illustrative examples of a recombinagenic oligonucleobase is found in following patent publications, which are incorporated in their entirety be reference herein: U.S. Patent Nos. 5,565,350; 5,756,325; 5,871,984; 5,760,012; 5,731,181; 5,888,983; 5,795,972; 5, 780,296; 5,945,339; 6,004,804; and 6,010,907 and in International Patent No. PCT/US00/23457; and in International Patent Publication Nos. WO 98/49350; WO 99/07865; WO 99/58723; WO 99/58702; and WO 99/40789.

The plant can be of any species of dicotyledonous, monocotyledonous or gymnospermous plant, including any woody plant species that grows as a tree or shrub, any herbaceous species, or any species that produces edible fruits, seeds or vegetables, or any

species that produces colorful or aromatic flowers. For example, the plant may be selected from a species of plant from the group consisting of canola, sunflower, tobacco, sugar beet, cotton, maize, wheat, barley, rice, sorghum, tomato, mango, peach, apple, pear, strawberry, banana, melon, potato, carrot, lettuce, onion, soya spp, sugar cane, pea, field beans, poplar, grape, citrus, alfalfa, rye, oats, turf and forage grasses, flax, oilseed rape, cucumber, morning glory, balsam, pepper, eggplant, marigold, lotus, cabbage, daisy, carnation, tulip, iris, lily, and nut producing plants insofar as they are not already specifically mentioned.

The recombinagenic oligonucleobase can be introduced into a plant cell using any method commonly used in the art, including but not limited to, microcarriers (biolistic delivery), microfibers, electroporation, microinjection.

The invention is also directed to the culture of cells mutated according to the methods of the present invention in order to obtain a plant that produces seeds, henceforth a "fertile plant", and the production of seeds and additional plants from such a fertile plant.

The invention is further directed to a method of selectively controlling weeds in a field, the field comprising plants with the disclosed EPSPS gene alterations and weeds, the method comprising application to the field of a herbicide to which the said plants have been rendered resistant.

The invention is also directed to novel mutations in the EPSPS gene that confer resistance or tolerance to a member of the phosphonomethylglycine family, *e.g.*, glyphosate, to a plant or wherein the mutated EPSPS has substantially the same enzymatic activity as compared to wild-type EPSPS.

3.1 DEFINITIONS

The invention is to be understood in accordance with the following definitions.

An oligonucleobase is a polymer of nucleobases, which polymer can hybridize by Watson-Crick base pairing to a DNA having the complementary sequence.

Nucleobases comprise a base, which is a purine, pyrimidine, or a derivative or analog thereof. Nucleobases include peptide nucleobases, the subunits of peptide nucleic acids, and morpholine nucleobases as well as nucleosides and nucleotides. Nucleosides are nucleobases that contain a pentosefuranosyl moiety, e.g., an optionally substituted riboside or 2'-deoxyriboside. Nucleosides can be linked by one of several linkage moieties, which may or may not contain a phosphorus. Nucleosides that are linked by unsubstituted phosphodiester linkages are termed nucleotides.

An oligonucleobase chain has a single 5' and 3' terminus, which are the ultimate nucleobases of the polymer. A particular oligonucleobase chain can contain

nucleobases of all types. An oligonucleobase compound is a compound comprising one or more oligonucleobase chains that are complementary and hybridized by Watson-Crick base pairing. Nucleobases are either deoxyribo-type or ribo-type. Ribo-type nucleobases are pentosefuranosyl containing nucleobases wherein the 2' carbon is a methylene substituted with a hydroxyl, alkyloxy or halogen. Deoxyribo-type nucleobases are nucleobases other than ribo-type nucleobases and include all nucleobases that do not contain a pentosefuranosyl moiety.

An oligonucleobase strand generically includes both oligonucleobase chains and segments or regions of oligonucleobase chains. An oligonucleobase strand has a 3' end and a 5' end. When a oligonucleobase strand is coextensive with a chain, the 3' and 5' ends of the strand are also 3' and 5' termini of the chain.

According to the present invention, substantially normal growth of a plant, plant organ, plant tissue or plant cell is defined as a growth rate or rate of cell division of the plant, plant organ, plant tissue, or plant cell that is at least 35%, at least 50%, at least 60%, or at least 75% of the growth rate or rate of cell division in a corresponding plant, plant organ, plant tissue or plant cell expressing the wild type EPSPS protein.

According to the present invention, substantially normal development of a plant, plant organ, plant tissue or plant cell is defined as the occurrence of one or more developmental events in the plant, plant organ, plant tissue or plant cell that are substantially the same as those occurring in a corresponding plant, plant organ, plant tissue or plant cell expressing the wild type EPSPS protein.

According to the present invention plant organs include, but are not limited to, leaves, stems, roots, vegetative buds, floral buds, meristems, embryos, cotyledons, endosperm, sepals, petals, pistils, carpels, stamens, anthers, microspores, pollen, pollen tubes, ovules, ovaries and fruits, or sections, slices or discs taken therefrom. Plant tissues include, but are not limited to, callus tissues, ground tissues, vascular tissues, storage tissues, meristematic tissues, leaf tissues, shoot tissues, root tissues, gall tissues, plant tumor tissues, and reproductive tissues. Plant cells include, but are not limited to, isolated cells with cell walls, variously sized aggregates thereof, and protoplasts.

Plants are substantially "tolerant" to glyphosate when they are subjected to it and provide a dose/response curve which is shifted to the right when compared with that provided by similarly subjected non-tolerant like plant. Such dose/response curves have "dose" plotted on the X-axis and "percentage kill", "herbicidal effect", etc., plotted on the y-axis. Tolerant plants will require more herbicide than non-tolerant like plants in order to produce a given herbicidal effect. Plants which are substantially "resistant" to the glyphosate exhibit few, if any, necrotic, lytic, chlorotic or other lesions, when subjected to

glyphosate at concentrations and rates which are typically employed by the agrochemical community to kill weeds in the field. Plants which are resistant to a herbicide are also tolerant of the herbicide. The terms "resistant" and "tolerant" are to be construed as "tolerant and/or resistant" within the context of the present application.

4. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A is the DNA sequence of *Arabidopsis thaliana* EPSPS gene (SEQ ID NO:1). The bold underlined nucleotide residues are the targeted residues.

FIG. 1B is the amino acid sequence of *Arabidopsis thaliana* EPSPS protein (SEQ ID NO:2). The bold and underlined amino acid residues are the targeted residues.

FIG. 2 is a list of the *Arabidopsis thaliana* wild-type and mutant EPSPS nucleotide and amino acid sequences in the region of amino acid position 173 to 183; wild-type nucleotide sequence (SEQ ID NO:1) and wild-type amino acid sequence (SEQ ID NO:2), mutant A_{177} nucleotide sequence (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4); mutant I_{178} nucleotide sequence (SEQ ID NO:5) and amino acid sequence (SEQ ID NO:6); mutant $A_{177}I_{178}$ nucleotide sequence (SEQ ID NO:7) and amino acid sequence (SEQ ID NO:8); mutant $I_{178}S_{182}$ nucleotide sequence (SEQ ID NO:9) and amino acid sequence (SEQ ID NO:10); mutant $A_{177}S_{182}$ nucleotide sequence (SEQ ID NO:11) and amino acid sequence (SEQ ID NO:12); mutant $A_{177}I_{178}S_{182}$ nucleotide sequence (SEQ ID NO:13) and amino acid sequence (SEQ ID NO:14); mutant $V_{177}S_{182}$ nucleotide sequence (SEQ ID NO:15) and amino acid sequence (SEQ ID NO:16); mutant $L_{178}S_{182}$ nucleotide sequence (SEQ ID NO:17) and amino acid sequence (SEQ ID NO:18); mutant $A_{177}V_{178}$ nucleotide sequence (SEQ ID NO:19) and amino acid sequence (SEQ ID NO:20); mutant $A_{177}L_{182}$ nucleotide sequence (SEQ ID NO:21) and amino acid sequence (SEQ ID NO:22).

FIG. 3A-C is an alignment of the DNA of *Arabidopsis thaliana* EPSPS gene performed by DNAStar (LaserGene), (SEQ ID NO:1) with the nucleotide sequences of *Brassica napus* (SEQ ID NO:23); *Petunia hybrida* (SEQ ID NO:24); and *Zea mays* (SEQ ID NO:25) EPSPS gene. The sequences are aligned using J. Hein method with weighted residue weight table.

FIG. 4 is an alignment of the *Arabidopsis thaliana* EPSPS amino acid sequence (SEQ ID NO:2) with the *Brassica napus* (SEQ ID NO:26); *Petunia hybrida* (SEQ ID NO:27); and *Zea mays* (SEQ ID NO:28) EPSPS amino acid sequences. The sequences are aligned using J. Hein method with weighted residue weight table.

FIG. 5 is a list of the mutagenesis primers used, with the targeted codons in bold characters (mutant primer A_{177} (SEQ ID NO:29); mutant primer I_{178} (SEQ ID NO:30);

mutant primer $A_{177}I_{178}$ (SEQ ID NO:31); mutant primer $I_{178}S_{182}$ (SEQ ID NO:32); mutant primer $A_{177}S_{182}$ (SEQ ID NO:34); mutant primer $A_{177}I_{178}S_{182}$ (SEQ ID NO:35); mutant primer $V_{177}S_{182}$ (SEQ ID NO:35); mutant primer $L_{178}S_{182}$ (SEQ ID NO:36); mutant primer $A_{177}V_{178}$ (SEQ ID NO:37); and mutant primer $A_{177}L_{182}$ (SEQ ID NO:38)).

FIG. 6 is the growth measured by optical density at 600 nm of *Arabidopsis* clones in the presence (+) and absence (-) of 17 mM glyphosate.

FIG. 7 (top panel) is a western blot showing the expression of His-tagged *Bacillus*, *Arabidopsis* wild type (WT) and mutant (AS) EPSPS proteins isolated from cell lysates (L) and eluates (E). Untransformed *Salmonella* as a negative control shows no EPSPS expression. The bottom panel is a silver-stained duplicate gel.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a non-transgenic plant or plant cell having a mutation in the EPSPS gene, which plant has increased resistance or tolerance to a member of the phosphonomethylglycine family and which plant exhibits substantially normal growth or development of the plant, its organs, tissues or cells, as compared to the corresponding wild-type plant or cell. The present invention is also directed to a non-transgenic plant having a mutation in the EPSPS gene, which plant is resistant to or has an increased tolerance to a member of the phosphonomethylglycine family, *e.g.*, glyphosate, wherein the mutated EPSPS protein has substantially the same catalytic activity as compared to the wild-type EPSPS protein.

The present invention is also directed to a method for producing a non-transgenic plant having a mutated EPSPS gene that substantially maintains the catalytic activity of the wild-type protein irrespective of the presence or absence of a herbicide of the phosphonomethylglycine family. The method comprises introducing into a plant cell a recombinagenic oligonucleobase with a targeted mutation in the EPSPS gene and identifying a cell, seed, or plant having a mutated EPSPS gene.

Illustrative examples of a recombinagenic oligonucleobase is found in following patent publications, which are incorporated in their entirety be reference herein: U.S. Patent Nos. 5,565,350; 5,756,325; 5,871,984; 5,760,012; 5,731,181; 5,888,983; 5,795,972; 5, 780,296; 5,945,339; 6,004,804; and 6,010,907 and in International Patent No. PCT/US00/23457; and in International Patent Publication Nos. WO 98/49350; WO 99/07865; WO 99/58723; WO 99/58702; and WO 99/40789.

The plant can be of any species of dicotyledonous, monocotyledonous or gymnospermous plant, including any woody plant species that grows as a tree or shrub, any herbaceous species, or any species that produces edible fruits, seeds or vegetables, or any

species that produces colorful or aromatic flowers. For example, the plant may be selected from a species of plant from the group consisting of canola, sunflower, tobacco, sugar beet, cotton, maize, wheat, barley, rice, sorghum, tomato, mango, peach, apple, pear, strawberry, banana, melon, potato, carrot, lettuce, onion, soya spp, sugar cane, pea, field beans, poplar, grape, citrus, alfalfa, rye, oats, turf and forage grasses, flax, oilseed rape, cucumber, morning glory, balsam, pepper, eggplant, marigold, lotus, cabbage, daisy, carnation, tulip, iris, lily, and nut producing plants insofar as they are not already specifically mentioned.

The recombinagenic oligonucleobase can be introduced into a plant cell using any method commonly used in the art, including but not limited to, microcarriers (biolistic delivery), microfibers, electroporation, microinjection.

The invention is also directed to the culture of cells mutated according to the methods of the present invention in order to obtain a plant that produces seeds, henceforth a "fertile plant", and the production of seeds and additional plants from such a fertile plant.

The invention is further directed to a method of selectively controlling weeds in a field, the field comprising plants with the disclosed EPSPS gene alterations and weeds, the method comprising application to the field of a herbicide to which the said plants have been rendered resistant.

The invention is also directed to novel mutations in the EPSPS gene that confer resistance or tolerance to a member of the phosphonomethylglycine family, *e.g.*, glyphosate, to a plant or wherein the mutated EPSPS has substantially the same enzymatic activity as compared to wild-type EPSPS.

5.1 RECOMBINAGENIC OLIGONUCLEOBASES

The invention can be practiced with recombinagenic oligonucleobases having the conformations and chemistries described in United States patent No. 5,565,350 to Kmiec (Kmiec I) and U.S. patent No. 5,731,181 (Kmiec II) gene, which are hereby incorporated by reference. Kmiec I teaches a method for introducing specific genetic alterations into a target gene. The recombinagenic oligonucleobases in Kmiec I and/or Kmiec II contain two complementary strands, one of which contains at least one segment of RNA-type nucleotides (an "RNA segment") that are base paired to DNA-type nucleotides of the other strand.

Kmiec II discloses that purine and pyrimidine base-containing non-nucleotides can be substituted for nucleotides. U.S. Patent Nos. 5,756,325; 5,871,984; 5,760,012; 5,888,983; 5,795,972; 5, 780,296; 5,945,339; 6,004,804; and 6,010,907 and in International Patent No. PCT/US00/23457; and in International Patent Publication Nos. WO 98/49350; WO 99/07865; WO 99/58723; WO 99/58702; and WO 99/40789, which are each

hereby incorporated in their entirety, disclose additional recombinagenic molecules that can be used for the present invention. The term "recombinagenic oligonucleobase" is used herein to denote the molecules that can be used in the methods of the present invention and include mixed duplex oligonucleotides, non-nucleotide containing molecules taught in Kmiec II, single stranded oligodeoxynucleotides and other recombinagenic molecules taught in the above noted patents and patent publications.

In one embodiment, the recombinagenic oligonucleobase is a mixed duplex oligonucleotide in which the RNA-type nucleotides of the mixed duplex oligonucleotide are made RNase resistant by replacing the 2'-hydroxyl with a fluoro, chloro or bromo functionality or by placing a substituent on the 2'-O. Suitable substituents include the substituents taught by the Kmiec II. Alternative substituents include the substituents taught by U.S. Patent No. 5,334,711 (Sproat) and the substituents taught by patent publications EP 629 387 and EP 679 657 (collectively, the Martin Applications), which are hereby incorporated by reference. As used herein, a 2' -fluoro, chloro or bromo derivative of a ribonucleotide or a ribonucleotide having a 2'-OH substituted with a substituent described in the Martin Applications or Sproat is termed a "2'-Substituted Ribonucleotide." As used herein the term "RNA-type nucleotide" means a 2'-hydroxyl or 2'-Substituted Nucleotide that is linked to other nucleotides of a mixed duplex oligonucleotide by an unsubstituted phosphodiester linkage or any of the non-natural linkages taught by Kmiec I or Kmiec II. As used herein the term "deoxyribo-type nucleotide" means a nucleotide having a 2'-H, which can be linked to other nucleotides of a MDON by an unsubstituted phosphodiester linkage or any of the non-natural linkages taught by Kmiec I or Kmiec II.

In a particular embodiment of the present invention, the recombinagenic oligonucleobase is a mixed duplex oligonucleotide that is linked solely by unsubstituted phosphodiester bonds. In alternative embodiments , the linkage is by substituted phosphodiesters, phosphodiester derivatives and non-phosphorus-based linkages as taught by Kmiec II. In yet another embodiment, each RNA-type nucleotide in the mixed duplex oligonucleotide is a 2'-Substituted Nucleotide. Particular preferred embodiments of 2'-Substituted Ribonucleotides are 2'-fluoro, 2'-methoxy, 2'-propyloxy, 2'-allyloxy, 2'-hydroxylethyloxy, 2'-methoxyethyloxy, 2'-fluoropropyloxy and 2'-trifluoropropyloxy substituted ribonucleotides. More preferred embodiments of 2'-Substituted Ribonucleotides are 2'-fluoro, 2'-methoxy, 2'-methoxyethyloxy, and 2'-allyloxy substituted nucleotides. In another embodiment the mixed duplex oligonucleotide is linked by unsubstituted phosphodiester bonds.

Although mixed duplex oligonucleotide having only a single type of 2'-substituted RNA-type nucleotide are more conveniently synthesized, the methods of the

invention can be practiced with mixed duplex oligonucleotides having two or more types of RNA-type nucleotides. The function of an RNA segment may not be affected by an interruption caused by the introduction of a deoxynucleotide between two RNA-type trinucleotides, accordingly, the term RNA segment encompasses such an "interrupted RNA segment." An uninterrupted RNA segment is termed a contiguous RNA segment. In an alternative embodiment an RNA segment can contain alternating RNase-resistant and unsubstituted 2'-OH nucleotides. The mixed duplex oligonucleotides preferably have fewer than 100 nucleotides and more preferably fewer than 85 nucleotides, but more than 50 nucleotides. The first and second strands are Watson-Crick base paired. In one embodiment the strands of the mixed duplex oligonucleotide are covalently bonded by a linker, such as a single stranded hexa, penta or tetranucleotide so that the first and second strands are segments of a single oligonucleotide chain having a single 3' and a single 5' end. The 3' and 5' ends can be protected by the addition of a "hairpin cap" whereby the 3' and 5' terminal nucleotides are Watson-Crick paired to adjacent nucleotides. A second hairpin cap can, additionally, be placed at the junction between the first and second strands distant from the 3' and 5' ends, so that the Watson-Crick pairing between the first and second strands is stabilized.

The first and second strands contain two regions that are homologous with two fragments of the target EPSPS gene, *i.e.*, have the same sequence as the target gene. A homologous region contains the nucleotides of an RNA segment and may contain one or more DNA-type nucleotides of connecting DNA segment and may also contain DNA-type nucleotides that are not within the intervening DNA segment. The two regions of homology are separated by, and each is adjacent to, a region having a sequence that differs from the sequence of the target gene, termed a "heterologous region." The heterologous region can contain one, two or three mismatched nucleotides. The mismatched nucleotides can be contiguous or alternatively can be separated by one or two nucleotides that are homologous with the target gene. Alternatively, the heterologous region can also contain an insertion or one, two, three or of five or fewer nucleotides. Alternatively, the sequence of the mixed duplex oligonucleotide may differ from the sequence of the target gene only by the deletion of one, two , three, or five or fewer nucleotides from the mixed duplex oligonucleotide. The length and position of the heterologous region is, in this case, deemed to be the length of the deletion, even though no nucleotides of the mixed duplex oligonucleotide are within the heterologous region. The distance between the fragments of the target gene that are complementary to the two homologous regions is identically the length of the heterologous region when a substitution or substitutions is intended. When the heterologous region contains an insertion, the homologous regions are thereby separated in

the mixed duplex oligonucleotide farther than their complementary homologous fragments are in the gene, and the converse is applicable when the heterologous region encodes a deletion.

The RNA segments of the mixed duplex oligonucleotides are each a part of a homologous region, *i.e.*, a region that is identical in sequence to a fragment of the target gene, which segments together preferably contain at least 13 RNA-type nucleotides and preferably from 16 to 25 RNA-type nucleotides or yet more preferably 18-22 RNA-type nucleotides or most preferably 20 nucleotides. In one embodiment, RNA segments of the homology regions are separated by and adjacent to, *i.e.*, "connected by" an intervening DNA segment. In one embodiment, each nucleotide of the heterologous region is a nucleotide of the intervening DNA segment. An intervening DNA segment that contains the heterologous region of a mixed duplex oligonucleotide is termed a "mutator segment."

The change to be introduced into the target EPSPS gene is encoded by the heterologous region. The change to be introduced into the EPSPS gene may be a change in one or more bases of the EPSPS gene sequence or the addition or deletion of one or more bases.

In another embodiment of the present invention, the recombinagenic oligonucleobase is a single stranded oligodeoxynucleotide mutational vector or SSOMV, which is disclosed in International Patent Application PCT/US00/23457, which is incorporated by reference in its entirety. The sequence of the SSOMV is based on the same principles as the mutational vectors described in U.S. Patent Nos. 5,756,325; 5,871,984; 5,760,012; 5,888,983; 5,795,972; 5, 780,296; 5,945,339; 6,004,804; and 6,010,907 and in International Publication Nos. WO 98/49350; WO 99/07865; WO 99/58723; WO 99/58702; and WO 99/40789. The sequence of the SSOMV contains two regions that are homologous with the target sequence separated by a region that contains the desired genetic alteration termed the mutator region. The mutator region can have a sequence that is the same length as the sequence that separates the homologous regions in the target sequence, but having a different sequence. Such a mutator region can cause a substitution. Alternatively, the homolgous regions in the SSOMV can be contiguous to each other, while the regions in the target gene having the same sequence are separated by one, two or more nucleotides. Such a SSOMV causes a deletion from the target gene of the nucleotides that are absent from the SSOMV. Lastly, the sequence of the target gene that is identical to the homologous regions may be adjacent in the target gene but separated by one two or more nucleotides in the sequence of the SSOMV. Such an SSOMV causes an insertion in the sequence of target gene.

The nucleotides of the SSOMV are deoxyribonucleotides that are linked by unmodified phosphodiester bonds except that the 3' terminal and/or 5' terminal internucleotide linkage or alternatively the two 3' terminal and/or 5' terminal internucleotide linkages can be a phosphorothioate or phosphoamidate. As used herein an internucleotide linkage is the linkage between nucleotides of the SSOMV and does not include the linkage between the 3' end nucleotide or 5' end nucleotide and a blocking substituent, see *supra*. In a specific embodiment the length of the SSOMV is between 21 and 55 deoxynucleotides and the lengths of the homology regions are, accordingly, a total length of at least 20 deoxynucleotides and at least two homology regions should each have lengths of at least 8 deoxynucleotides.

The SSOMV can be designed to be complementary to either the coding or the non-coding strand of the target gene. When the desired mutation is a substitution of a single base, it is preferred that both the mutator nucleotide be a pyrimidine. To the extent that is consistent with achieving the desired functional result it is preferred that both the mutator nucleotide and the targeted nucleotide in the complementary strand be pyrimidines. Particularly preferred are SSOMV that encode transversion mutations, *i.e.*, a C or T mutator nucleotide is mismatched, respectively, with a C or T nucleotide in the complementary strand.

In addition to the oligodeoxynucleotide the SSOMV can contain a 5' blocking substituent that is attached to the 5' terminal carbons through a linker. The chemistry of the linker is not critical other than its length, which should preferably be at least 6 atoms long and that the linker should be flexible. A variety of non-toxic substituents such as biotin, cholesterol or other steroids or a non-intercalating cationic fluorescent dye can be used. Particularly preferred as reagents to make SSOMV are the reagents sold as Cy3™ and Cy5™ by Glen Research, Sterling VA, which are blocked phosphoroamidites that upon incorporation into an oligonucleotide yield 3,3,3',3'-tetramethyl N,N'-isopropyl substituted indomonocarbocyanine and indodicarbocyanine dyes, respectively. Cy3 is the most preferred. When the indocarbocyanine is N-oxyalkyl substituted it can be conveniently linked to the 5' terminal of the oligodeoxynucleotide through as a phosphodiester with a 5' terminal phosphate. The chemistry of the dye linker between the dye and the oligodeoxynucleotide is not critical and is chosen for synthetic convenience. When the commercially available Cy3 phosphoramidite is used as directed the resulting 5' modification consists of a blocking substituent and linker together which are a N-hydroxypropyl, N'-phosphatidylpropyl 3,3,3',3'-tetramethyl indomonocarbocyanine.

In the preferred embodiment the indocarbocyanine dye is tetra substituted at the 3 and 3' positions of the indole rings. Without limitation as to theory these substitutions

prevent the dye from being an intercalating dye. The identity of the substituents at these positions are not critical. The SSOMV can in addition have a 3' blocking substituent. Again the chemistry of the 3' blocking substituent is not critical.

5.2 THE LOCATION AND TYPE OF MUTATION INTRODUCED INTO THE EPSPS GENE

In one embodiment of the present invention, the *Arabidopsis thaliana* EPSPS gene (see Figure 1A) and corresponding EPSPS enzyme (see Figure 1B) comprises a mutation at one or more amino acid residues selected from the group consisting of Leu_{173}, Gly_{177}, Thr_{178}, Ala_{179}, Met_{180}, Arg_{181}, Pro_{182}, Ser_{98}, Ser_{255} and Leu_{198}, or at an analogous position in an EPSPS paralog, and the mutation results in one or more of the following amino acid substitutions in the EPSPS enzyme in comparison with the wild-type sequence:

(i) Leu_{173} - Phe
(ii) Gly_{177} - Ala or Ile
(iii) Thr_{178} - Ile or Val or Leu
(iv) Ala_{179} - Gly
(v) Met_{180} - Cys
(vi) Arg_{181} - Leu or Ser
(vii) Pro_{182} - Leu or Ser
(viii) Ser_{98} -Asp
(ix) Ser_{255} -Ala
(x) Leu_{198} -Lys.

In another embodiment of the present invention, within the EPSPS gene product, the amino acid residue to be changed is Leu within the contiguous sequence Leu-Tyr-Leu-Gly-Asn (SEQ ID NO:29) and is changed to Phe; or the amino acid residue to be changed is Gly within the contiguous sequence Asn-Ala-Gly-Thr-Ala (SEQ ID NO:30) and is changed to Ala or Ile; or the amino acid to be changed is Thr within the contiguous sequence Ala-Gly-Thr-Ala-Met (SEQ ID NO:31) and is changed to Ile, Val or Leu; or the amino acid to be changed is Ala within the contiguous sequence Gly-Thr-Ala-Met-Arg (SEQ ID NO:32) and is changed to Gly; or the amino acid to be changed is Met within the contiguous sequence Thr-Ala-Met-Arg-Pro (SEQ ID NO:33) and is changed to Cys; or the amino acid to be changed is Arg within the contiguous sequence Ala-Met-Arg-Pro-Leu (SEQ ID NO:34) and is changed to Leu or Ser; or the amino acid to be changed is Pro within the contiguous sequence Met-Arg-Pro-Leu-Thr (SEQ ID NO:35) and is changed to Leu or Ser; or the amino acid to be changed is Ser within a contiguous Pro-Gly-Ser-Lys-Ser (SEQ ID NO:36) and is changed to Asp; or the amino acid to be changed is Ser within the

contiguous sequence Ile-Ser-Ser-Gln-Tyr (SEQ ID NO:37) and is changed to Ala; or the amino acid to be changed is Leu within the contiguous sequence Tyr-Val-Leu-Asp-Gly (SEQ ID NO:38) and is changed to Lys. In other embodiments, one or more of the foregoing changes can be made in the EPSPS amino acid sequence.

Alternatively, and/or additionally, the mutation may result in the replacement of any amino acid at positions corresponding to 256, 284-288 and 353-356 with respect to the EPSPS protein depicted in Figure 1B (SEQ ID NO. 2).

In specific embodiments of the present invention, the EPSPS gene is mutated at amino acid position 177 in which Gly is replaced by Ala. Another specific embodiment is the substitution of Thr at amino acid position 178 by Ile. A further specific embodiment comprises a mutation at amino acid position 177 in which Gly is replaced by Ala, plus the additional substitution of Thr at amino acid position 178 by Ile. Other specific embodiments of the present invention are directed to mutations at amino acid position 178, in which Thr is replaced by Ile, plus the additional mutation at position 182, in which Pro is replaced by Ser. Other embodiments include the substitution of Gly at amino acid position 177 by Ala, plus the additional mutation at amino acid position 182, in which Pro is substituted by Ser. Other mutated EPSPS sequences comprise the substitution of Gly at position 177 by Ala, plus the substitution at position 178, in which Thr is replaced by Ile, plus the additional substitution of Pro at amino acid position 182 by Ser. Another embodiment is the substitution of Thr at amino acid position 178 by Val and the additional mutation at amino acid position182, in which Pro is replaced by Ser. A further specific embodiment includes the substitution of Thr at position 178 by Leu, plus the mutation at amino acid position 182, in which Pro is replaced by Ser. A further embodiment includes, the substitution at amino acid position 177 in which Gly is replaced by Ala, plus the substitution of Thr at position 178 by Val. The invention also embodies the substitution at amino acid position 177 in which Gly is replaced by Ala, plus the replacement of Thr at amino acid position 178 by Leu (see Figure 2).

The foregoing mutations in the EPSPS gene were described using the *Arabidopsis thaliana* EPSPS gene (SEQ ID NO:1) and protein (SEQ ID NO:2). The present invention also encompasses mutant EPSPS genes of other species (paralogs). However, due to variations in the EPSPS genes of different species, the number of the amino acid residue to be changed in one species may be different in another species. Nevertheless, the analogous position is readily identified by one of skill in the art by sequence homology. For example, Figure 3A-C shows the aligned nucleotide sequences and Figure 4 shows the aligned amino acid sequences of four paralogs of the EPSPS gene, *Arabidopsis thaliana*, *Zea mays*, *Petunia hybrida*, and *Brassica napus*. Thus, the analogous

positions in *Zea mays* are Leu_{97}, Gly_{101}, Thr_{102}, Ala_{103}, Met_{104}, Arg_{105}, Pro_{106}, Ser_{23}, Ser_{179} and Leu_{122}. Thus, the *Zea mays* EPSPS amino acid sequence is mutated at one or more of the following amino acid positions and results in one or more of the following substitutions:

(i) Leu_{97} - Phe
(ii) Gly_{101} - Ala or Ile
(iii) Thr_{102} - Ile or Val or Leu
(iv) Ala_{103} - Gly
(v) Met_{104} - Cys
(vi) Arg_{105} - Leu or Ser
(vii) Pro_{106} - Leu or Ser
(viii) Ser_{23} -Asp
(ix) Ser_{179} -Ala
(x) Leu_{122} -Lys.

In another embodiment of the present invention, within the *Zea mays* EPSPS gene product the amino acid residue to be changed is Leu within the contiguous sequence Leu-Phe-Leu-Gly-Asn (SEQ ID NO:39) and is changed to Phe; or the amino acid residue to be changed is Gly within the contiguous sequence Asn-Ala-Gly-Thr-Ala (SEQ ID NO:30) and is changed to Ala or Ile; or the amino acid to be changed is Thr within the contiguous sequence Ala-Gly-Thr-Ala-Met (SEQ ID NO:31) and is changed to Ile, Val or Leu; or the amino acid to be changed is Ala within the contiguous sequence Gly-Thr-Ala-Met-Arg (SEQ ID NO:32) and is changed to Gly; or the amino acid to be changed is Met within the contiguous sequence Thr-Ala-Met-Arg-Pro (SEQ ID NO:33) and is changed to Cys; or the amino acid to be changed is Arg within the contiguous sequence Ala-Met-Arg-Pro-Leu (SEQ ID NO:34) and is changed to Leu or Ser; or the amino acid to be changed is Pro within the contiguous sequence Met-Arg-Pro-Leu-Thr (SEQ ID NO:35) and is changed to Leu or Ser; or the amino acid to be changed is Ser within a contiguous Pro-Gly-Ser-Lys-Ser (SEQ ID NO:36) and is changed to Asp; or the amino acid to be changed is Ser within the contiguous sequence Ile-Ser-Ser-Gln-Tyr (SEQ ID NO:37) and is changed to Ala; or the amino acid to be changed is Leu within the contiguous sequence Tyr-Val-Leu-Asp-Gly (SEQ ID NO:38) and is changed to Lys. In other embodiments, one or more of the foregoing changes can be made in the EPSPS amino acid sequence.

In *Brassica napus*, the analogous amino acid positions are Leu_{169}, Gly_{173}, Thr_{174}, Ala_{175}, Met_{176}, Arg_{177}, Pro_{178}, Ser_{94}, Ser_{251} and Leu_{194}. Thus, the *Brassica napus* EPSPS amino acid sequence is mutated at one or more of the following amino acid positions and results in one or more of the following substitutions:

(i) Leu_{169} - Phe

(ii) Gly_{173} - Ala or Ile
(iii) Thr_{174} - Ile or Val or Leu
(iv) Ala_{175} - Gly
(v) Met_{176} - Cys
(vi) Arg_{177} - Leu or Ser
(vii) Pro_{178} - Leu or Ser
(viii) Ser_{94} -Asp
(ix) Ser_{251} -Ala
(x) Leu_{194} -Lys

In another embodiment of the present invention, within the *Brassica napus* EPSPS gene product the amino acid residue to be changed is Leu within the contiguous sequence Leu-Tyr-Leu-Gly-Asn (SEQ ID NO:29) and is changed to Phe; or the amino acid residue to be changed is Gly within the contiguous sequence Asn-Ala-Gly-Thr-Ala (SEQ ID NO:30) and is changed to Ala or Ile; or the amino acid to be changed is Thr within the contiguous sequence Ala-Gly-Thr-Ala-Met (SEQ ID NO:31) and is changed to Ile, Val or Leu; or the amino acid to be changed is Ala within the contiguous sequence Gly-Thr-Ala-Met-Arg (SEQ ID NO:32) and is changed to Gly; or the amino acid to be changed is Met within the contiguous sequence Thr-Ala-Met-Arg-Pro (SEQ ID NO:33) and is changed to Cys; or the amino acid to be changed is Arg within the contiguous sequence Ala-Met-Arg-Pro-Leu (SEQ ID NO:34) and is changed to Leu or Ser; or the amino acid to be changed is Pro within the contiguous sequence Met-Arg-Pro-Leu-Thr (SEQ ID NO:35) and is changed to Leu or Ser; or the amino acid to be changed is Ser within a contiguous Pro-Gly-Ser-Lys-Ser (SEQ ID NO:36) and is changed to Asp; or the amino acid to be changed is Ser within the contiguous sequence Ile-Ser-Ser-Gln-Tyr (SEQ ID NO:37) and is changed to Ala; or the amino acid to be changed is Leu within the contiguous sequence Tyr-Val-Leu-Asp-Gly (SEQ ID NO:38) and is changed to Lys. In other embodiments, one or more of the foregoing changes can be made in the EPSPS amino acid sequence.

In *Petunia hybrida* the analogous positions are Leu_{169}, Gly_{173}, Thr_{174}, Ala_{175}, Met_{176}, Arg_{177}, Pro_{178}, Ser_{94}, Ser_{251} and Leu_{194}. Thus, the *Petunia hybrida* EPSPS amino acid sequence is mutated at one or more of the following amino acid positions and results in one or more of the following substitutions:

(i) Leu_{169} - Phe
(ii) Gly_{173} - Ala or Ile
(iii) Thr_{174} - Ile or Val or Leu
(iv) Ala_{175} - Gly
(v) Met_{176} - Cys

(vi) Arg_{177} - Leu or Ser

(vii) Pro_{178} - Leu or Ser

(viii) Ser_{94} -Asp

(ix) Ser_{251} -Ala

(x) Leu_{194} -Lys

In another embodiment of the present invention, within the *Petunia hybrida* EPSPS gene product the amino acid residue to be changed is Leu within the contiguous sequence Leu-Phe-Leu-Gly-Asn (SEQ ID NO:39) and is changed to Phe; or the amino acid residue to be changed is Gly within the contiguous sequence Asn-Ala-Gly-Thr-Ala (SEQ ID NO:30) and is changed to Ala or Ile; or the amino acid to be changed is Thr within the contiguous sequence Ala-Gly-Thr-Ala-Met (SEQ ID NO:31) and is changed to Ile, Val or Leu; or the amino acid to be changed is Ala within the contiguous sequence Gly-Thr-Ala-Met-Arg (SEQ ID NO:32) and is changed to Gly; or the amino acid to be changed is Met within the contiguous sequence Thr-Ala-Met-Arg-Pro (SEQ ID NO:33) and is changed to Cys; or the amino acid to be changed is Arg within the contiguous sequence Ala-Met-Arg-Pro-Leu (SEQ ID NO:34) and is changed to Leu or Ser; or the amino acid to be changed is Pro within the contiguous sequence Met-Arg-Pro-Leu-Thr (SEQ ID NO:35) and is changed to Leu or Ser; or the amino acid to be changed is Ser within a contiguous Pro-Gly-Ser-Lys-Ser (SEQ ID NO:36) and is changed to Asp; or the amino acid to be changed is Ser within the contiguous sequence Ile-Ser-Ser-Gln-Tyr (SEQ ID NO:37) and is changed to Ala; or the amino acid to be changed is Leu within the contiguous sequence Tyr-Val-Leu-Asp-Gly (SEQ ID NO:38) and is changed to Lys. In other embodiments, one or more of the foregoing changes can be made in the EPSPS amino acid sequence.

5.3 THE DELIVERY OF RECOMBINAGENIC OLIGONUCLEOBASES INTO PLANT CELLS

Any commonly known method can be used in the methods of the present invention to transform a plant cell with a recombinagenic oligonucleobases. Illustrative methods are listed below.

5.3.1 MICROCARRIERS AND MICROFIBERS

The use of metallic microcarriers (microspheres) for introducing large fragments of DNA into plant cells having cellulose cell walls by projectile penetration is well known to those skilled in the relevant art (henceforth biolistic delivery). United States Patent Nos. 4,945,050; 5,100,792 and 5,204,253 describe general techniques for selecting microcarriers and devices for projecting them.

Specific conditions for using microcarriers in the methods of the present invention are described in International Publication WO 99/07865. In an illustrative technique, ice cold microcarriers (60 mg/ml), mixed duplex oligonucleotide (60 mg/ml) 2.5 M $CaCl_2$ and 0.1 M spermidine are added in that order; the mixture gently agitated, *e.g.*, by vortexing, for 10 minutes and let stand at room temperature for 10 minutes, whereupon the microcarriers are diluted in 5 volumes of ethanol, centrifuged and resuspended in 100% ethanol. Good results can be obtained with a concentration in the adhering solution of 8-10 μg/μl microcarriers, 14-17 μg/ml mixed duplex oligonucleotide, 1.1-1.4 M $CaCl_2$ and 18-22 mM spermidine. Optimal results were observed under the conditions of 8 μg/μl microcarriers, 16.5 μg/ml mixed duplex oligonucleotide, 1.3 M $CaCl_2$ and 21 mM spermidine.

Recombinagenic oligonucleobases can also be introduced into plant cells for the practice of the present invention using microfibers to penetrate the cell wall and cell membrane. U.S. Patent No. 5,302,523 to Coffee et al. describes the use of 30 x 0.5 μm and 10 x 0.3 μm silicon carbide fibers to facilitate transformation of suspension maize cultures of Black Mexican Sweet. Any mechanical technique that can be used to introduce DNA for transformation of a plant cell using microfibers can be used to deliver recombinagenic oligonucleobases for transmutation.

An illustrative technique for microfiber delivery of a recombinagenic oligonucleobase is as follows: Sterile microfibers (2 μg) are suspended in 150 μl of plant culture medium containing about 10 μg of a mixed duplex oligonucleotide. A suspension culture is allowed to settle and equal volumes of packed cells and the sterile fiber/nucleotide suspension are vortexed for 10 minutes and plated. Selective media are applied immediately or with a delay of up to about 120 hours as is appropriate for the particular trait.

5.3.2 PROTOPLAST ELECTROPORATION

In an alternative embodiment, the recombinagenic oligonucleobases can be delivered to the plant cell by electroporation of a protoplast derived from a plant part. The protoplasts are formed by enzymatic treatment of a plant part, particularly a leaf, according to techniques well known to those skilled in the art. *See, e.g.*, Gallois et al., 1996, in Methods in Molecular Biology 55:89-107, Humana Press, Totowa, NJ; Kipp et al., 1999, in Methods in Molecular Biology 133:213-221, Humana Press, Totowa, NJ. The protoplasts need not be cultured in growth media prior to electroporation. Illustrative conditions for electroporation are 3×10^5 protoplasts in a total volume of 0.3 ml with a concentration of recombinagenic oligonucleobase of between 0.6 - 4 μg/mL.

5.3.3 WHISKERS AND MICROINJECTION

In yet another alternative embodiment, the recombinagenic oligonucleobase can be delivered to the plant cell by whiskers or microinjection of the plant cell. The so called whiskers technique is performed essentially as described in Frame et al., 1994, Plant J. 6:941-948. The recombinagenic oligonucleobase is added to the whiskers and used to transform the plant cells. The recombinagenic oligonucleobase may be co-incubated with plasmids comprising sequences encoding proteins capable of forming recombinase complexes in plant cells such that recombination is catalyzed between the oligonucleotide and the target sequence in the EPSPS gene.

5.4 SELECTION OF GLYPHOSATE RESISTANT PLANTS

Plants or plant cells can be tested for resistance or tolerance to a herbicide using commonly known methods in the art, *e.g.*, by growing the plant or plant cell in the presence of a herbicide and measuring the rate of growth as compared to the growth rate in the absence of the herbicide.

6. EXAMPLE

The following experiments demonstrate the production of mutant *Arabidopsis thaliana* EPSPS genes which are resistant to the herbicide glyphosate and which allows the plant cells to maintain a growth rate

6.1 MATERIAL AND METHODS

6.1.1 ISOLATION OF *ARABIDOPSIS THALIANA* EPSPS cDNA

A 1.3 kb DNA fragment was amplified by PCR from an *Arabidopsis* cDNA library using the primers AtEXPEXPM1 and AtEXPEXP2CM-2. The two primers were designed to amplify the cDNA from the mature peptide to the termination codon. The 5' primer AtEXPEXPM1 contains an XbaI site (underlined) and the 3' primer AtEXPEXP2CM-2 contains a BglII site (underlined), sites which will be of use for cloning of the fragment into the expression vector.

AtEXPEXPM1
5'-GC<u>TCTAGA</u>GAAAGCGTCGGAGATTGTACTT-3' (SEQ ID NO:40)

AtEXPEXP2CM-2
5'-GC<u>AGATCT</u>GAGCTCTTAGTGCTTTGTGATTCTTTCAAGTAC-3' (SEQ ID NO:41)

The PCR band was excised from the agarose gel and purified (GeneClean, Biol). Its sequence was then confirmed as the mature peptide sequence of *Arabidopsis thaliana* EPSPS gene.

6.1.2 PREPARATION OF THE EXPRESSION VECTOR

The EPSPS coding region of the *AroE Bacillus subtilis* gene was obtained by PCR using the following primers:

BsAroE5'Xba

5'-GCGTCTAGAAAAACGAGATAAGGTGCAG-3' (SEQ ID NO:42) and

BsAroE3'BamHI

5'-GCGGATCCTCAGGATTTTTCGAAAGCTTATTTAAATG-3' (SEQ ID NO:43).

The PCR fragment, lacking an initiation codon (ATG), was cloned in-frame to the pACLacIMH6RecA vector by replacing the ORF of *RecA* by digesting with XbaI and BamHI. PACLacIMH6RecA contained the LacI region of Pet21 at positions 1440 to 3176, the MH6 RecA at positions 3809 to 5188, chloramphenicol resistance gene at positions 5445-218 (5446 to 5885 and 1 to 218), and the p15A origin of replication at positions 581 to 1424. The coding region of *RecA* gene was cloned from *E.coli* in-frame with the start codon and 6 histidine linker (MH6) behind the LacZ promoter of pUC19.

6.1.3 CLONING OF THE ARABIDOPSIS EPSPS GENE INTO BACTERIAL EXPRESSION VECTOR

The *Arabidopsis* 1.3 kb PCR fragment was digested with XbaI and BamHI (compatible with BglII) and cloned into the plasmid pACYCLacIMH6EPSPS, in place of the *Bacillus* gene.

The clones obtained (selected on chloramphenicol) were then sequenced and confirmed positive. One of the confirmed clones (pAtEPS-12) was selected and the junctions between the cDNA and the cloning plasmid were also confirmed to be identical to the expected sequences.

6.1.4 NOVEL POINT MUTATIONS IN THE EPSPS GENE

Ten different mutants of the *Arabidopsis thaliana* EPSPS gene were designed, (see Figure 2). For the mutagenesis experiments, PCR primers were designed with one, two or three mutations. The PCR reactions were performed using a regular

flanking primer (5'ATEPS-198: 5'- GAAAGCGTCGGAGATTGTAC-3' (SEQ ID NO:44)) and one of the mutation-carrying primers (see Figure 5).

The 353bp PCR fragments obtained were purified (Qiagen PCR Purification kit) and their sequence confirmed. The fragments were then digested with PstI (underlined in the primer sequences) and BamHI and ligated to the pAtEPS-12 vector, which had itself been previously digested with PstI and BamHI.JM109 (Promega) competent cells were used for the transformation and plated onto chloramphenicol-containing LB plates. Clones from each mutagenesis experiment were then isolated and their sequence confirmed.

6.1.5 GLYPHOSATE RESISTANCE ASSAYS

Electrocompetent cells of SA4247, a LacZ - *Salmonella typhi* strain, were prepared according to well known procedures (see Current Protocols in Molecular Biology, (Wiley and Sons, Inc.)). 30 μl of SA4247 competent cells were electroporated with 20 ng of each plasmid DNA encoding *Arabidopsis* wild-type and mutant EPSPS proteins, *Bacillus* wild-type EPSPS, along with a mock transfection as a control. The settings for electroporation were 25 μF, 2.5KV and 200 ohms. After electroporation, the cells were transferred into 15 mls culture tube and supplemented with 970 μl of SOC medium. The cultures were incubated for 1 ½ hours at 37°C at 225 rpm. 50 μl of each culture were plated onto LB plates containing 17 μg/ml chloramphenicol (in duplicates) and incubated overnight at 37°C. On the following day, 5 colonies of each plate were picked and transferred onto M9 plates and incubated overnight at 37°C.

Colonies from the overnight incubation on solid M9 were inoculated into 4 ml of liquid M9 medium and grown overnight at 37°C. On the following day, 25 ml of liquid M9 medium containing chloramphenicol, IPTG and 17 mM or 0 mM Glyphosate (Aldrich, 33775-7) were inoculated with 1-2 mls of each overnight culture (in duplicates), the starting OD (at 600 nm) was measured and all the cultures were normalized to start at the same OD. An OD measurement was taken every hour for seven hours. As a control of the bacterial growth, a culture of untransformed *Salmonella* was also inoculated into plain LB medium. In two independent experiments, the clones $A_{177}I_{178}$, $A_{177}V_{178}$, $A_{177}L_{178}$ and I_{177} did not grow in M9 medium, therefore the glyphosate-resistance assays could not be performed on them.

6.1.7 ISOLATION AND PURIFICATION OF THE EXPRESSED PROTEIN FROM BACTERIAL CLONES

One milliliter of overnight culture of each of the bacterial clones is inoculated into 100 ml of liquid LB medium containing chloramphenicol. The cells were

allowed to grow at 37°C until they reached an OD of 0.5-0.7 (approximately 3 ½ hours). IPTG was then added to the cultures to a concentration of 1.0 mM. The cells were grown five additional hours. They were then pelleted at 4000 rpm for 20 minutes at 4°C.

The isolation and the purification of the His-tagged proteins were performed following the Qiagen Ni-NTA Protein Purification System. Cell lysates and eluates were run in duplicates on 12.5% acrylamide gels. One of the gels was silver-stained for immediate visualization, the second gel was transferred onto Millipore Immobilon-P membrane, and blocked overnight in 5% milk in TBS-T. The membrane was then exposed to Anti-His primary antibody solution (Amersham Pharmacia biotech, cat# 37-4710), followed by exposure to Anti-Mouse-IgG secondary antibody solution. (NIF825, from Amersham Pharmacia biotech ECLWestern blotting anlysis system, cat# RPN2108). Washes and detection reactions were performed according to the manufacturer instructions. Autoradiograms were developed after 5 minutes exposure.

6.2 RESULTS

Cells containing a mutation in the EPSPS gene produced cells that were both resistant to the herbicide glyphosate and that had a substantially similar growth rate in the absence or presence of glyphosate, as compared to the wild-type cells, irrespective of the presence of glyphosate (see Figure 6).

It was also demonstrated that the *Arabidopsis* clones containing a mutant EPSPS gene expressed the mutant protein at substantially the same level as the wild-type protein (see Figure 7).

The invention claimed and described herein is not to be limited in scope by the specific embodiments, including but not limited to the deposited microorganism embodiments, herein disclosed since these embodiments are intended as illustrations of several aspects of the invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

A number of references are cited herein, the entire disclosures of which are incorporated herein, in their entirety, by reference.

WE CLAIM:

1. A non-transgenic herbicide resistant plant, which plant expresses a mutant EPSPS gene product and which plant has substantially normal growth as compared to a plant expressing the wild-type EPSPS gene product.

2. A non-transgenic herbicide resistant plant, which plant expresses a mutant EPSPS gene product, which gene product has substantially the same level of catalytic activity as compared to the wild-type gene product.

3. The plant according to claim 1 or 2 in which the herbicide is a member of the phosphonomethylglycine family.

4. The plant according to claim 3 in which the member of the phosphonomethylglycine family is glyphosate.

5. The plant according to claim 1 or 2 in which the EPSPS gene is mutated at one or more amino acid positions, said positions selected from the group consisting of Leu_{173}, Gly_{177}, Thr_{178}, Ala_{179}, Met_{180}, Arg_{181}, Pro_{182}, Ser_{98}, Ser_{255} and Leu_{198} in *Arabidopsis* or at an analogous amino acid residue in an EPSPS paralog.

6. The plant according to claim 5 in which the positions in the *Zea mays* paralog are selected from the group consisting of Leu_{97}, Gly_{101}, Thr_{102}, Ala_{103}, Met_{104}, Arg_{105}, Pro_{106}, Ser_{23}, Ser_{179} and Leu_{122}.

7. The plant according to claim 5 in which the positions in the *Brassica napus* paralog are selected from the group consisting of Leu_{169}, Gly_{173}, Thr_{174}, Ala_{175}, Met_{176}, Arg_{177}, Pro_{178}, Ser_{94}, Ser_{251} and Leu_{194}.

8. The plant according to claim 5 in which the positions in the *Petunia hybrida* are selected from the group consisting of Leu_{169}, Gly_{173}, Thr_{174}, Ala_{175}, Met_{176}, Arg_{177}, Pro_{178}, Ser_{94}, Ser_{251} and Leu_{194}.

9. The plant according to claim 1 or 2 in which the plant is selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape,

canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

10. The plant according to claim 5 in which the mutated gene results in one or more of the following amino acid substitutions in the EPSPS enzyme in comparison with the wild-type sequence:

(i) Leu_{173} - Phe
(ii) Gly_{177} - Ala or Ile
(iii) Thr_{178} - Ile or Val or Leu
(iv) Ala_{179} - Gly
(v) Met_{180} - Cys
(vi) Arg_{181} - Leu or Ser
(vii) Pro_{182} - Leu or Ser
(viii) Ser_{98} -Asp
(ix) Ser_{255} -Ala
(x) Leu_{198} -Lys.

11. The plant according to claim 6 in which the mutated gene results in one or more of the following amino acid substitutions in the EPSPS enzyme in comparison with the wild-type sequence:

(i) Leu_{97} - Phe
(ii) Gly_{101} - Ala or Ile
(iii) Thr_{102} - Ile or Val or Leu
(iv) Ala_{103} - Gly
(v) Met_{104} - Cys
(vi) Arg_{105} - Leu or Ser
(vii) Pro_{106} - Leu or Ser
(viii) Ser_{23} -Asp
(ix) Ser_{179} -Ala
(x) Leu_{122} -Lys.

12. The plant according to claim 7 in which the mutated gene results in one or more of the following amino acid substitutions in the EPSPS enzyme in comparison with the wild-type sequence:

(i) Leu_{169} - Phe
(ii) Gly_{173} - Ala or Ile

(iii) Thr_{174} - Ile or Val or Leu
(iv) Ala_{175} - Gly
(v) Met_{176} - Cys
(vi) Arg_{177} - Leu or Ser
(vii) Pro_{178} - Leu or Ser
(viii) Ser_{94} -Asp
(ix) Ser_{251} -Ala
(x) Leu_{194} -Lys.

13. The plant according to claim 8 in which the mutated gene results in one or more of the following amino acid substitutions in the EPSPS enzyme in comparison with the wild-type sequence:

(i) Leu_{169} - Phe
(ii) Gly_{173} - Ala or Ile
(iii) Thr_{174} - Ile or Val or Leu
(iv) Ala_{175} - Gly
(v) Met_{176} - Cys
(vi) Arg_{177} - Leu or Ser
(vii) Pro_{178} - Leu or Ser
(viii) Ser_{94} -Asp
(ix) Ser_{251} -Ala
(x) Leu_{194} -Lys.

14. A method for producing a non-transgenic herbicide resistant or tolerant plant comprising

a. introducing into a plant cell a recombinagenic oligonucleobase to produce a mutant EPSPS gene; and

b. identifying a cell having a mutated EPSPS gene, which cell has substantially normal growth as compared to a corresponding wild-type plant cell.

15. A method for producing a non-transgenic herbicide resistant or tolerant plant comprising

a. introducing into a plant cell a recombinagenic oligonucleobase to produce a mutant EPSPS gene; and

b. identifying a cell having a mutated EPSPS gene, which encoded mutant EPSPS protein has substantially the same catalytic activity as compared to a corresponding wild-type EPSPS protein.

16. The method according to claim 14 or 15 in which the recombinagenic oligonucleobase is a mixed duplex nucleotide or a SSMOV.

17. The method according to claim 16 in which the mixed duplex nucleotide contains a first homologous region which has a sequence identical to the sequence of at least 6 base pairs of the first fragment of the target EPSPS gene and a second homologous region which has a sequence identical to the sequence of at least 6 based pairs of a second fragment of the target EPSPS gene, and an intervening region which contains at least one nucleobase heterologous to the target EPSPS gene, which intervening region connects the first and second homologous region.

18. The method according to claim 14 or 15 in which the recombinagenic oligonucleobase is introduced by electroporation.

19. The method according to claim 14 or 15 which the mutant EPSPS gene is mutated at one or more amino acid positions, said positions selected from the group consisting of Leu_{173}, Gly_{177}, Thr_{178}, Ala_{179}, Met_{180}, Arg_{181}, Pro_{182}, Ser_{98}, Ser_{255} and Leu_{198} in *Arabidopsis* or at an analogous amino acid residue in an EPSPS paralog.

20. The plant according to claim 19 in which the positions in the *Zea mays* paralog are selected from the group consisting of Leu_{97}, Gly_{101}, Thr_{102}, Ala_{103}, Met_{104}, Arg_{105}, Pro_{106}, Ser_{23}, Ser_{179} and Leu_{122}.

21. The plant according to claim 19 in which the positions in the *Brassica napus* paralog are selected from the group consisting of Leu_{169}, Gly_{173}, Thr_{174}, Ala_{175}, Met_{176}, Arg_{177}, Pro_{178}, Ser_{94}, Ser_{251} and Leu_{194}.

22. The plant according to claim 19 in which the positions in the *Petunia hybrida* are selected from the group consisting of Leu_{169}, Gly_{173}, Thr_{174}, Ala_{175}, Met_{176}, Arg_{177}, Pro_{178}, Ser_{94}, Ser_{251} and Leu_{194}.

23. The plant according to claim 14 or 15 in which the plant is selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape, turf grasses and *Brassica* sp.

24. An isolated mutant EPSPS protein comprising the amino acid sequence depicted in SEQ ID NO:2, in which amino acid position Leu_{173} is replaced with Phe, Gly_{177} is replaced with Ala or Ile, Thr_{178} is replaced with Ile or Val or Leu, Ala_{179} is replaced with Gly, Met_{180} is replaced with Cys, Arg_{181} is replaced with Leu or Ser, Pro_{182} is replaced with Leu or Ser, Ser_{98} is replaced with Asp, Ser_{255} is replaced with Ala or Leu_{198} is replaced with Lys, which mutant EPSPS protein has increased resistance or tolerance to a herbicide, which herbicide is a member of the phosphonomethylglycine family.

DNA sequence:

cccttcatgtcttttgtagaaaccccattatctttcttagggcccaattgaaaacccacattttctttcacctaaccca
ccaaagccttgcacatgttgacgtgaacaccaaactaacacgtgtcatactgccagtggttatgataaatgctcatacc
ataccagagtcatagagtttttggttggtgaaagatttgacggatgccttcttctcatttctcaccaactccctccaaa
cccaacaaaatgtttatattagcaaagccgccaaagtgtaaacgaaagtttataaatttcatttctgtgatcttacgta
attggaggaagatcaaaattttcaatccccattcttcgattgcttcaattgaagtttctccg

[transit peptide start]

ATGGCGCAAGTTAGCAGAATCTGCAATGGTGTGCAGAACCCATCTCTTATCTCCAATCTCTCGAAATCCAGTCAACGCA
AATCTCCCTTATCGGTTTCTCTGAAGACGCAGCAGCATCCACGAGCTTATCCGATTTCGTCGTCGTGGGGATTGAAGAA
GAGTGGGATGACGTTAATTGGCTCTGAGCTTCGTCCTCTTAAGGTCATGTCTTCTGTTTCCACGGCGGAG

[mature peptide starts]

AAAGCGTCGGAGATTGTACTTCAACCCATTAGAGAAATCTCCGGTCTTATTAAGCTTCCTGGCTCCAAGTCTCTATCAA
ATCGGATCCTGCTTCTCGCTGCTCTGTCTGAGGTATATATCACTTCGTTTCGTCCTTCTCTGTAATCTGAACTTAGATT
ATAAAGATTGATACTTTACCATTTTGCTGTGGTTTTATAGGGAACAACTGTAGTGGACAACTTGTTGAATAGCGATGAC
ATCAATTACATGCTTGATGCGTTGAAGAGATTGGGACTTAATGTGGAAACTGACAGTGAAAATAATCGTGCTGTAGTTG
AAGGATGTGGCGGGATATTCCCAGCTTCCATAGATTCAAAGAGTGATATCGAACTTTACCTCGGTAATGCAGGAACAGC
AATGCGTCCACTTACCGCTGCGGTCACTGCTGCAGGTGGAAACGCAAGGTAGATTGAAGGAGTTGATGCTTCTTGGTAT
TTGATGTTTAAGGAATGGAGCTTTTGTTGATGCTTTATGATCCATTTATTCCAGTTATGTGCTTGATGGGGTGCCTCGT
ATGAGAGAAAGACCTATAGGGGATTTGGTTGTTGGTCTTAAGCAGCTTGGTGCTGATGTTGAATGTACTCTTGGAACTA
ACTGCCCTCCTGTTCGTGTCAACGCTAATGGTGGCCTTCCCGGTGGAAAGGTTAGATCTTGCAAATGGCATGTGAATAT
GTAATCTCGTTCCTTACTCTATGAACACTTGCAGAAATGTGTGTTCATCATAGCCTTAGCTTGACAAGATTTCAGTTTT
TAATCTACTCTCAACGGATGGATCCTAAAATAGAATCGGATTTGGTGATTGGTTTTCGTTCTCGATTACCGTTTTCGTT
GTATGATTTCTTGATTAACAATTAGGAGACATGTTATGCATTTGCAGGTGAAGCTTTCTGGATCAATTAGTAGTCAGTA
CTTGACTGCTCTGCTCATGTCTGCTCCCTTAGCTCTTGGAGACGTCGAGATTGAGATTGTCGATAAATTAATTTCTGTT
CCATATGTTGAAATGACATTGAAGTTGATGGAACGTTTCGGGGTTAGTGTCGAGCATAGTGATAGCTGGGATCGTTTCT
TTGTCAAGGGCGGGCAAAAATACAAGTAGGAGTTATTCTTTTCTTCCTTTTCTGAAATCACATCCCTTAGCTTGACAAT
ATAATGACTAAAAGGTGAATGATTCAGGTCTCCGGGTAATGCGTATGTAGAAGGTGATGCTTCTAGTGCATGTTATTTC
TTGGCTGGTGCTGCCATTACCGGTGAAACTGTCACAGTCGAAGGTTGTGGAACTACCAGCTTGCAGGTAATATTTGTAC
ACTGAATCATCGACGAGGCTGTTAAGTTTATAGTGAAATTCGTCTAGGTCAAAGTTTCATCTTTTGACAAGTTGTATAT
AACATATTCGCAAGATTCTAAGCTCAATTTTTGTGATGAATCTCTAGGGAGATGTAAAATTCGCCGAGGTCCTTGAGAA
AATGGGATGTAAAGTGTCCTGGACAGAGAACAGTGTGACTGTGACAGGACCACCTAGAGATGCTTTTGGAATGAGACAC
TTGCGGGCTATTGATGTCAACATGAACAAAATGCCTGATGTAGCCATGACCCTTGCCGTCGTTGCTCTCTTTGCTGACG
GTCCAACCACCATTAGAGATGGTAAGTAAAAAGCTCTCTCTTATAATTAAGGTTTCTCAATATTCATGATCACTTAATT
CTGTTTGGTTAATATAGTGGCTAGCTGGAGAGTAAAGGAGACAGAAAGGATGATTGCCATTTGCACAGAGCTTAGAAAA
GTAAGAGATTCTTATCTCTCTCTTTCTGTCTCTTGACAGTGCTCATTCTAAGTAATTAGCTCATAAATTTGTGTGTTTG
TGTTCAGCTGGGAGCTACAGTGGAAGAAGGTTCAGATTATTGTGTGATAACTCCGCCCAAAAAGGTGAAAACGGCAGAG
ATTGATACATATGATGATCATAGAATGGCAATGGCATTCTCTCTTGCAGCTTGTGCTGATGTTCCAATCACCATCAACG
ACTCTGGTTGCACCAGGAAAACCTTCCCCGACTACTTCCAAGTACTTGAAAGAATCACAAAGCAC**TAA**acaataaactc
tgtttttcttctgatccaagctt

FIG.1A

<u>Protein sequence:</u>

MAQVSRICNGVQNPSLISNLSKSSQRKSPLSVSLKTQQHPRAYPISSSWGLKKSGMTLIGSELRPLKVMSSVSTAE
KASEIVLQPIREISGLIKLPGSKSLSNRILLLAALSEGTTVVDNLLNSDDINYMLDALKRLGLNVETDSENNRAVV
EGCGGIFPASIDSKSDIELYLGNA<u>GT</u>AMR<u>P</u>LTAAVTAAGGNASYVLDGVPRMRERPIGDLVVGLKQLGADVECTLG
TNCPPVRVNANGGLPGGKVKLSGSISSQYLTALLMSAPLALGDVEIEIVDKLISVPYVEMTLKLMERFGVSVEHSD
SWDRFFVKGGQKYKSPGNAYVEGDASSACYFLAGAAITGETVTVEGCGTTSLQGDVKFAEVLEKMGCKVSWTENSV
TVTGPPRDAFGMRHLRAIDVNMNKMPDVAMTLAVVALFADGPTTIRDVASWRVKETERMIAICTELRKLGATVEEG
SDYCVITPPKKVKTAEIDTYDDHRMAMAFSLAACADVPITINDSGCTRKTFPDYFQVLERITKH

FIG.1B

__Arabidopsis thaliana wild type sequence:__

Position	173	174	175	176	177	178	179	180	181	182	183
	L	*G*	*N*	*A*	*G*	*T*	*A*	*M*	*R*	*P*	*L*
	CTC	GGT	AAT	GCA	*GGA*	*ACA*	GCA	ATG	CGT	*CCA*	CTT

__Arabidopsis thaliana mutant sequences:__

Name											
A_{177}	CTC	GGT	AAT	GCA	**GCA**	ACA	GCA	ATG	CGT	CCA	CTT
	L	*G*	*N*	*A*	*A*	*T*	*A*	*M*	*R*	*P*	*L*
I_{178}	CTC	GGT	AAT	GCA	GCA	*ATA*	GCA	ATG	CGT	CCA	CTT
	L	*G*	*N*	*A*	*G*	*I*	*A*	*M*	*R*	*P*	*L*
$A_{177}I_{178}$	CTC	GGT	AAT	GCA	*GCA*	*ATA*	GCA	ATG	CGT	CCA	CTT
	L	*G*	*N*	*A*	*A*	*I*	*A*	*M*	*R*	*P*	*L*
$I_{178}S_{182}$	CTC	GGT	AAT	GCA	GGA	*ATA*	GCA	ATG	CGT	*TCA*	CTT
	L	*G*	*N*	*A*	*G*	*I*	*A*	*M*	*R*	*S*	*L*
$A_{177}S_{182}$	CTC	GGT	AAT	GCA	*GCA*	ACA	GCA	ATG	CGT	*TCA*	CTT
	L	*G*	*N*	*A*	*A*	*T*	*A*	*M*	*R*	*S*	*L*
$A_{177}I_{178}S_{182}$	CTC	GGT	AAT	GCA	*GCA*	*ATA*	GCA	ATG	CGT	*TCA*	CTT
	L	*G*	*N*	*A*	*A*	*I*	*A*	*M*	*R*	*S*	*L*
$V_{178}S_{182}$	CTC	GGT	AAT	GCA	GGA	*GTA*	GCA	ATG	CGT	*TCA*	CTT
	L	*G*	*N*	*A*	*G*	*V*	*A*	*M*	*R*	*S*	*L*
$L_{178}S_{182}$	CTC	GGT	AAT	GCA	GGA	*TTA*	GCA	ATG	CGT	*TCA*	CTT
	L	*G*	*N*	*A*	*G*	*L*	*A*	*M*	*R*	*S*	*L*
$A_{177}V_{178}$	CTC	GGT	AAT	GCA	*GCA*	*GTA*	GCA	ATG	CGT	CCA	CTT
	L	*G*	*N*	*A*	*A*	*V*	*A*	*M*	*R*	*P*	*L*
$A_{177}L_{178}$	CTC	GGT	AAT	GCA	*GCA*	*TTA*	GCA	ATG	CGT	CCA	CTT
	L	*G*	*N*	*A*	*A*	*L*	*A*	*M*	*R*	*P*	*L*

FIG.2

4/10

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           10        20        30        40          50        60        70        80           90
  1 ATGGCGCAAGTTAGCAGAATCTGCAATGGTGTGCAGAACCCAT---CTCTTATCTCCAATCTCTCGAAATCCAGTCAACGCAAATCTCC---CTTATCGG atepspscDNA.SEQ
  1 ATGGCGCAATCTAGCAGAATCTGCCATGGCGTGCAGAACCCATGTGTTATCATCTCCAATCTCTCCAAATCCAACCAAAACAAATCACC---TTTCTCCG bnepscDNA.SEQ
  1 ATGGCACAAATTAACAACATGGCTCAAGGGATACAAACCCTTA---ATCCCAATTCCAATTTCCATAAACCCCAAGTTCCTAAATCTTCAAGTTTTCTTG petaroacDNA.SEQ
  1 GCGG------------------------------------------------------------------------------------------ zmepsps.SEQ

       100       110       120       130       140       150       160       170       180       190
 95 TTTCT---CTGAAGACGCAGCAGCATCCACGAGCTTATCCGATTTCGTCGTCGTGGGGATTGAAGAAGAGTGGGATGACGTTAATTGGCTCTGAGCTTCG atepspscDNA.SEQ
 98 TCTCC---TTGAAGACGCATCAGC---CTCGAGCTT-----------CTTCGTGGGGATTGAAGAAGAGTGGAACGATGCTAAACGGTTCTGTAATTCG bnepscDNA.SEQ
 98 TTTTTGGATCTAAAAAACTGAAAAATTCAGCAAATT-----------CTATGTTGGTTTTGAAAAAAGATTCAATTTT------TATGCAAAAGTTTTG petaroacDNA.SEQ
  5 -----------------------------------------------------------------------------------GTGCCGAGG----- zmepsps.SEQ

       200       210       220       230       240       250       260       270       280       290
192 TCCTCTTAAGGTCATGTCTTCTGTTTCCACGGCGGAGAAAGCGTCGGAGATTGTACTTCAACCCATTAGAGAAATCTCCGGTCTTATTAAGCTTCCTGGC atepspscDNA.SEQ
180 CCCGGTTAAGGTAACAGCTTCTGTTTCCACGTCCGAGAAAGCTTCAGAGATTGTGCTTCAACCAATCAGACAAATCTCGGGTCTCATTAAGCTACCCGGA bnepscDNA.SEQ
180 TTCCTTTAGGATTTCAGCATCAGTGGCTACAGCACAGAAGCCTTCTGAGATAGTGTTGCAACCCATTAAAGAGATTTCAGGCACTGTTAAATTGCCTGGC petaroacDNA.SEQ
 14 -----------------------------------------------AGATCGTGCTGCAGCCCATCAAGGAGATCTCCGGCACCGTCAAGCTGCCGGGG zmepsps.SEQ

       300       310       320       330       340       350       360       370       380       390
292 TCCAAGTCTCTATCAAATCGGATCCTGCTTCTCGCTGCTCTGTCTGAGGGAACAACTGTAGTGGACAACTTGTTGAATAGCGATGACATCAATTACATGC atepspscDNA.SEQ
280 TCCAAATCTCTCTCCAATCGGATCCTCCTTCTTGCCGCTCTATCTGAGGGAACTACTGTAGTGGACAACTTGTTGAACAGTGATGACATCAACTACATGC bnepscDNA.SEQ
280 TCTAAATCATTATCTAATAGAATTCTCCTTCTTGCTGCCTTATCTGAAGGAACAACTGTGGTTGACAATTTACTAAGTAGTGATGATATTCATTACATGC petaroacDNA.SEQ
 67 TCCAAGTCGCTTTCCAACCGGATCCTCCTACTCGCCGCCCTGTCCGAGGGGACAACAGTGGTTGATAACCTGCTGAACAGTGAGGATGTCCACTACATGC zmepsps.SEQ

       400       410       420       430       440       450       460       470       480       490
392 TTGATGCGTTGAAGAGATTGGGACTTAATGTGGAAACTGACAGTGAAAATAATCGTGCTGTAGTTGAAGGATGTGGCGGGATATTCCCAGCTTCCATAGA atepspscDNA.SEQ
380 TTGATGCGTTGAAGAAGCTGGGGCTTAACGTGGAACGTGACAGTGTAAACAACCGTGCGGTTGTTGAAGGATGCGGTGGAATATTCCCAGCTTCCTTAGA bnepscDNA.SEQ
380 TTGGTGCCTTGAAAACACTTGGACTGCATGTAGAAGAAGATAGTGCAAACCAACGAGCTGTTGTTGAAGGTTGTGGTGGGCTTTTCCCTGTTGGTAAAGA petaroacDNA.SEQ
167 TCGGGGCCTTGAGGACTCTTGGTCTCTCTGTCGAAGCGGACAAAGCTGCCAAAAGAGCTGTAGTTGTTGGCTGTGGTGGAAAGTTCCCAGTTG---AGGA zmepsps.SEQ
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FIG.3A

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          500       510       520       530       540       550       560       570       580       590
492 TTCAAAGAGTGATATCGAACTTTACCTCGGTAATGCAGGAACAGCAATGCGTCCACTTACCGCTGCGGTCACTGCTGCAGGTGGAAACGCAAGTTATGTG atepspscDNA.SEQ
480 TTCCAAGAGTGATATTGAGTTGTACCTTGGGAATGCAGGAACAGCCATGCGTCCACTCACCGCTGCAGTTACAGCTGCAGGTGGCAACGCGAGTTATGTA bnepscDNA.SEQ
480 GTCCAAGGAAGAAATTCAACTGTTCCTTGGAAATGCAGGAACAGCAATGCGGCCACTAACAGCAGCAGTTACTGTAGCTGGTGGAAATTCAAGGTATGTA petaroacDNA.SEQ
264 TGCTAAAGAGGAAGTGCAGCTCTTCTTGGGGAATGCTGGAACTGCAATGCGGCCATTGACAGCAGCTGTTACTGCTGCTGGTGGAAATGCAACTTACGTG zmepsps.SEQ

          600       610       620       630       640       650       660       670       680       690
592 CTTGATGGGGTGCCTCGTATGAGAGAAAGACCTATAGGGGATTTGGTTGTTGGTCTTAAGCAGCTTGGTGCTGATGTTGAATGTACTCTTGGAACTAACT atepspscDNA.SEQ
580 CTTGATGGGGTGCCTAGAATGAGGGAAAGACCTATAGGAGATTTGGTTGTTGGTCTTAAGCAGCTTGGTGCTGATGTTGAGTGTACTCTTGGCACTAACT bnepscDNA.SEQ
580 CTTGATCGAGTTCCTCGAATGAGAGAGAGACCAATTAGTGATTTGGTTGATGGTCTTAAACAGCTTGGTGCAGAGGTTGATTGTTTCCTTGGTACGAAAT petaroacDNA.SEQ
364 CTTGATGGAGTACCAAGAATGAGGGAGAGACCCATTGGCGACTTGGTTGTCGGATTGAAGCAGCTTGGTGCAGATGTTGATTGTTTCCTTGGCACTGACT zmepsps.SEQ

          700       710       720       730       740       750       760       770       780       790
692 GCCCTCCTGTTCGTGTCAACGCTAATGGTGGCCTTCCCGGTGGAAAGGTGAAGCTTTCTGGATCAATTAGTAGTCAGTACTTGACTGCTCTGCTCATGTC atepspscDNA.SEQ
680 GTCCTCCTGTTCGTGTCAATGCTAATGGTGGCCTTCCCGGTGGAAAGGTGAAGCTTTCTGGATCGATCAGTAGTCAGTACTTGACTGCCCTCCTCATGGC bnepscDNA.SEQ
680 GTCCTCCTGTTCGAATTGTCAGCAAGGGAGGTCTTCCTGGAGGGAAGGTCAAGCTCTCTGGATCCATTAGCAGCCAATACTTGACTGCTCTGCTTATGGC petaroacDNA.SEQ
464 GCCCACCTGTTCGTGTCAATGGAATCGGAGGGCTACCTGGTGGCAAGGTCAAGCTGTCTGGCTCCATCAGCAGTCAGTACTTGAGTGCCTTGCTGATGGC zmepsps.SEQ

          800       810       820       830       840       850       860       870       880       890
792 TGCTCCCTTAGCTCTTGGAGACGTCGAGATTGAGATTGTCGATAAATTAATTTCTGTTCCATATGTTGAAATGACATTGAAGTTGATGGAACGTTTCGGG atepspscDNA.SEQ
780 AGCTCCTTTAGCTCTTGGAGACGTGGAGATTGAGATCATTGATAAACTGATATCTGTTCCATATGTTGAAATGACATTGAAGTTGATGGAGCGTTTTGGT bnepscDNA.SEQ
780 TGCTCCACTGGCTTTAGGAGATGTGGAGATTGAAATCATTGACAAACTAATTAGTGTACCTTATGTCGAGATGACATTGAAGTTGATGGAGCGATTTGGT petaroacDNA.SEQ
564 TGCTCCTTTGGCTCTTGGGGATGTGGAGATTGAAATCATTGATAAATTAATCTCCATTCCGTACGTCGAAATGACATTGAGATTGATGGAGCGTTTTGGT zmepsps.SEQ

          900       910       920       930       940       950       960       970       980       990
892 GTTAGTGTCGAGCATAGTGATAGCTGGGATCGTTTCTTTGTCAAGGGCGGGCAAAAATACAAGTCTCCGGGTAATGCGTATGTAGAAGGTGATGCTTCTA atepspscDNA.SEQ
880 GTTAGTGCCGAGCATAGTGATAGCTGGGATCGTTTCTTTGTCAAGGGCGGTCAGAAATACAAGTCGCCTGGTAATGCTTATGTAGAAGGTGATGCTTCTA bnepscDNA.SEQ
880 ATTTCTGTGGAGCACAGTAGTAGCTGGGACAGGTTCTTTGTCCGAGGAGGTCAGAAATACAAGTCTCCTGGAAAAGCTTTTGTCGAAGGTGATGCTTCAA petaroacDNA.SEQ
664 GTGAAAGCAGAGCATTCTGATAGCTGGGACAGATTCTACATTAAGGGAGGTCAAAAATACAAGTCCCCTAAAAATGCCTATGTTGAAGGTGATGCCTCAA zmepsps.SEQ
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FIG.3B

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        1000      1010      1020      1030      1040      1050      1060      1070      1080      1090
992  GTGCATGTTATTTCTTGGCTGGTGCTGCCATTACCGGTGAAACTGTCACAGTCGAAGGTTGTGGAACTACCAGCTTGCAGGGAGATGTAAAATTCGCCGA atepspscDNA.SEQ
980  GTGCTAGCTATTTCTTGGCTGGTGCTGCCATTACTGGTGAAACTGTTACTGTCGAAGGTTGTCGAACAACTAGCCTCCAGGGAGATGTGAAATTCGCAGA bnepscDNA.SEQ
980  GTGCTAGCTACTTCTTGGCTGGTGCAGCAGTCACAGGTGGAACTATCACTGTTGAAGGTTCTGGGACAAACAGTTTACAGGGGGATGTCAAATTTGCTGA petaroacDNA.SEQ
764  GCGCAAGCTATTTCTTGGCTGGTGCTGCAATTACTGGAGGGACTGTGACTGTGGAAGGTTGTGGCACCACCAGTTTGCAGGGTGATGTGAAGTTTGCTGA zmepsps.SEQ

        1100      1110      1120      1130      1140      1150      1160      1170      1180      1190
1092 GGTCCTTGAGAAAATGGGATGTAAAGTGTCCTGGACAGAGAACAGTGTGACTGTGACAGGACCACCTAGAGATGCTTTTGGAATGAGACACTTGCGGGCT atepspscDNA.SEQ
1080 GGTTCTTGAGAAAATGGGATGTAAAGTGTCATGGACAGAGAACAGTGTGACTGTGACTGGACCATCAAGAGATGCTTTTGGAATGAGGCACTTGCGTGGT bnepscDNA.SEQ
1080 GGTACTTGAAAAAATGGGAGCTGAAGTTACGTGGACAGAGAACAGTGTCACAGTCAAAGGACCTCCAAGGAGTTCTTCTGGGAGGAAGCATTTGCGTGCC petaroacDNA.SEQ
864  GGTACTGGAGATGATGGGAGCGAAGGTTACATGGACCGAGACTAGCGTAACTGTTACTGGCCCACCGCGGGAGCCATTTGGGAGGAAACACCTCAAGGCG zmepsps.SEQ

        1200      1210      1220      1230      1240      1250      1260      1270      1280      1290
1192 ATTGATGTCAACATGAACAAAATGCCTGATGTAGCCATGACCCTTGCCGTCGTTGCTCTCTTTGCTGACGGTCCAACCACCATTAGAGATGTGGCTAGCT atepspscDNA.SEQ
1180 GTTGATGTCAACATGAACAAAATGCCTGATGTAGCCATGACTCTAGCCGTTGTTGCTCTCTTTGCCGATGGTCCAACCACCATCAGAGATGTGGCTAGCT bnepscDNA.SEQ
1180 ATTGATGTGAACATGAATAAAATGCCTGATGTTGCCATGACACTTGCTGTTGTTGCACTTTATGCTGATGGTCCCACAGCTATAAGAGATGTTGCTAGCT petaroacDNA.SEQ
964  ATTGATGTCAACATGAACAAGATGCCTGATGTCGCCATGACTCTTGCTGTGGTTGCCCTCTTTGCCGATGGCCCGACAGCCATCAGAGACGTGGCTTCCT zmepsps.SEQ

        1300      1310      1320      1330      1340      1350      1360      1370      1380      1390
1292 GGAGAGTAAAGGAGACAGAAAGGATGATTGCCATTTGCACAGAGCTTAGAAAACTGGGAGCTACAGTGGAAGAAGGTTCAGATTATTGTGTGATAACTCC atepspscDNA.SEQ
1280 GGAGAGTTAAGGAGACAGAGAGGATGATTGCCATTTGCACAGAGCTTAGAAAGCTTGGAGCTACAGTGGAAGAAGGTTCAGATTATTGTGTGATAACTCC bnepscDNA.SEQ
1280 GGAGAGTCAAGGAAACTGAGCGCATGATCGCCATATGCACAGAACTTAGGAAGTTAGGAGCAACCGTTGAAGAAGGACCAGACTACTGCATAATCACCCC petaroacDNA.SEQ
1064 GGAGAGTAAAGGAGACCGAGAGGATGGTTGCGATCCGGACGGAGCTAACCAAGCTGGGAGCATCTGTTGAGGAAGGGCCGGACTACTGCATCATCACGCC zmepsps.SEQ

        1400      1410      1420      1430      1440      1450      1460      1470      1480      1490
1392 GCCCAAAAAGGTGAAAACGGCAGAGATTGATACATATGATGATCATAGAATGGCAATGGCATTCTCTCTTGCAGCTTGTGCTGATGTTCCAATCACCATC atepspscDNA.SEQ
1380 ACCAGCAAAGGTGAAACCGGCGGAGATTGATACGTATGATGATCATAGAATGGCGATGGCGTTCTCGCTTGCAGCTTGTGCTGATGTTCCAGTCACCATC bnepscDNA.SEQ
1380 ACCGGAGAAACTAAATGTGACCGATATTGATACATACGATGATCACAGGATGGCCATGGCTTTTTCTCTTGGTGCTTGTGCAGATGTTCCCGTCACCATC petaroacDNA.SEQ
1164 GCCGGAGAAGCTGAACGTGACGGCCATCGACACGTACGACGACCACAGGATGGCCATGGCCTTCTCCCTTGCCGCCTGTGCCGACGTCCCCGTCACCATC zmepsps.SEQ

        1500      1510      1520      1530      1540      1550      1560
1492 AACGACTCTGGTTGCACCAGGAAAACCTTCCCCGACTACTTCCAAGTACTTGAAAGAATCACAAAGGACTAA atepspscDNA.SEQ
1480 AAGGATCCTGGCTGCACCAGGAAGACTTTCCCTGACTACTTCCAAGTCCTTGAAAGTATCACAAAGCATTAA bnepscDNA.SEQ
1480 AATGACCCTGGCTGCACGCGGAAAACCTTCCCTAACTACTTTGATGTACTTCAGCAGTACTCCAAGGATTGA petaroacDNA.SEQ
1264 CGGGACCCTGGGTGCACCCGGAAGACCTTCCCCGACTACTTCGATGTGCTGAGCACTTTCGTCAAGAATTAA zmepsps.SEQ
```

FIG.3C

```
         10        20        30        40        50        60              70        80        90        100
  1 MAQVSRICNGVQNP-SLISNLSKSSQRKSPLSVSLKTQQHPRAYPISSSWGLKKSGMTLIGSELR------PLKVMSSVSTAEKASEIVLQPIREISGLIKLPGSKSLSN atepsps.PRO
  1 MAQSSRICHGVQNPCVTISNLSKSNQNKSPFSVSLKTHQ----PRASSWGLKKSGTMLNGSVIR------PVKVTASVSTSEKASEIVLQPIREISGLIKLPGSKSLSN bnepsps.PRO
  1 MAQINNMAQGIQTL-NPNSNFHKPQVPKSSSFLVFGSKK-----LKNSA----NSMLVLKKDSIFMQKFCSFRISASVATAQKPSEIVLQPIKEISGTVKLPGSKSLSN petaroa.PRO
  1 AG-----------------------------------------------------------------------------AEEIVLQPIKEISGTVKLPGSKSLSN zmepsps.PRO

         110       120       130       140       150       160       170       180       190       200       210
104 RILLLAALSEGTTVVDNLLNSDDINYMLDALKRLGLNVETDSENNRAVVEGCGGIFPASIDSKSDIELYLGNAGTAMRPLTAAVTAAGGNASYVLDGVPRMRERPICDLV atepsps.PRO
100 RILLLAALSEGTTVVDNLLNSDDINYMLDALKKLGLNVERDSVNNRAVVEGCGGIFPASLDSKSDIELYLGNAGTAMRPLTAAVTAAGGNASYVLDGVPRMRERPIGDLV bnepsps.PRO
100 RILLLAALSEGTTVVDNLLSSDDIHYMLGALKTLGLHVEEDSANQRAVVEGCGGLFPVGKESKEEIQLFLGNAGTAMRPLTAAVTVAGGNSRYVLDGVPRMRERPISDLV petaroa.PRO
 29 RILLLAALSEGTTVVDNLLNSEDVHYMLGALRTLGLSVEADKAAKRAVVVGCGGKFPV-EDAKEEVQLFLGNAGTAMRPLTAAVTAAGGNATYVLDGVPRMRERPIGDLV zmepsps.PRO

         220       230       240       250       260       270       280       290       300       310       320
214 VGLKQLGADVECTLGTNCPPVRVNANGGLPGGKVKLSGSISSQYLTALLMSAPLALGDVEIEIVDKLISVPYVEMTLKLMERFGVSVEHSDSWDRFFVKGGQKYKSPGNA atepsps.PRO
210 VGLKQLGADVECTLGTNCPPVRVNANGGLPGGKVKLSGSISSQYLTALLMAAPLALGDVEIEIIDKLISVPYVEMTLKLMERFGVSAEHSDSWDRFFVKGGQKYKSPGNA bnepsps.PRO
210 DGLKQLGAEVDCFLGTKCPPVRIVSKGGLPGGKVKLSGSISSQYLTALLMAAPLALGDVEIEIIDKLISVPYVEMTLKLMERFGISVEHSSSWDRFFVRGGQKYKSPGKA petaroa.PRO
138 VGLKQLGADVDCFLGTDCPPVRVNGIGGLPGGKVKLSGSISSQYLSALLMAAPLALGDVEIEIIDKLISIPYVEMTLRLMERFGVKAEHSDSWDRFYIKGGQKYKSPKNA zmepsps.PRO

         330       340       350       360       370       380       390       400       410       420       430
324 YVEGDASSACYFLAGAAITGETVTVEGCGTTSLQGDVKFAEVLEKMGCKVSWTENSVTVTGPPRDAFGMRHLRAIDVNMNKMPDVAMTLAVVALFADGPTTIRDVASWRV atepsps.PRO
320 YVEGDASSASYFLAGAAITGETVTVEGCGTTSLQGDVKFAEVLEKMGCKVSWTENSVTVTGPSRDAFGMRHLRAVDVNMNKMPDVAMTLAVVALFADGPTTIRDVASWRV bnepsps.PRO
320 FVEGDASSASYFLAGAAVTGGTITVEGCGTNSLQGDVKFAEVLEKMGAEVTWTENSVTVKGPPRSSSGRKHLRAIDVNMNKMPDVAMTLAVVALYADGPTAIRDVASWRV petaroa.PRO
248 YVEGDASSASYFLAGAAITGGTVTVEGCGTTSLQGDVKFAEVLEMMGAKVTWTETSVTVTGPPREPFGRKHLKAIDVNMNKMPDVAMTLAVVALFADGPTAIRDVASWRV zmepsps.PRO

         440       450       460       470       480       490       500       510       520
434 KETERMIAICTELRKLGATVEEGSDYCVITPPKKVKTAEIDTYDDHRMAMAFSLAACADVPITINDSGCTRKTFPDYFQVLERITKH atepsps.PRO
430 KETERMIAICTELRKLGATVEEGSDYCVITPPAKVKPAEIDTYDDHRMAMAFSLAACADVPVTIKDPGCTRKTFPDYFQVLESITKH bnepsps.PRO
430 KETERMIAICTELRKLGATVEEGPDYCIITPPEKLNVTDIDTYDDHRMAMAFSLAACADVPVTINDPGCTRKTFPNYFDVLQQYSKH petaroa.PRO
358 KETERMVAIRTELTKLGASVEEGPDYCIITPPEKLNVTAIDTYDDHRMAMAFSLAACAEVPVTIRDPGCTRKTFPDYFDVLSTFVKN zmepsps.PRO
```

FIG.4

Oligo Name	Oligo Sequence (5'→3')
ATEPS-A_{177}	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAGTGGACGCATTGCTGTT**GC**TGCATTACCGAG
ATEPS-AI	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAGTGGACGCATTGC**TATTGC**TGCATTACCGAG
ATEPS-IS	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAG**TGA**ACGCATTGC**TAT**TCCTGCATTACCGAG
ATEPS-AS	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAG**TGA**ACGCATTGCTGTT**GC**TGCATTACCGAG
ATEPS-AIS	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAG**TGA**ACGCATTGC**TATTGC**TGCATTACCGAG
ATEPS-I_{177}	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAGTGGACGCATTGCTGTT**AT**TGCATTACCGAG
ATEPS-VS	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAG**TGA**ACGCATTGC**TAC**TCCTGCATTACCGAG
ATEPS-LS	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAG**TGA**ACGCATTGC**TAA**TCCTGCATTACCGAG
ATEPS-AV	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAGTGGACGCATTGC**TACTGC**TGCATTACCGAG
ATEPS-AL	CGTTTCCACCTGCAGCAGTGACCGCAGCGGTAAGTGGACGCATTGC**TAATGC**TGCATTACCGAG

FIG.5

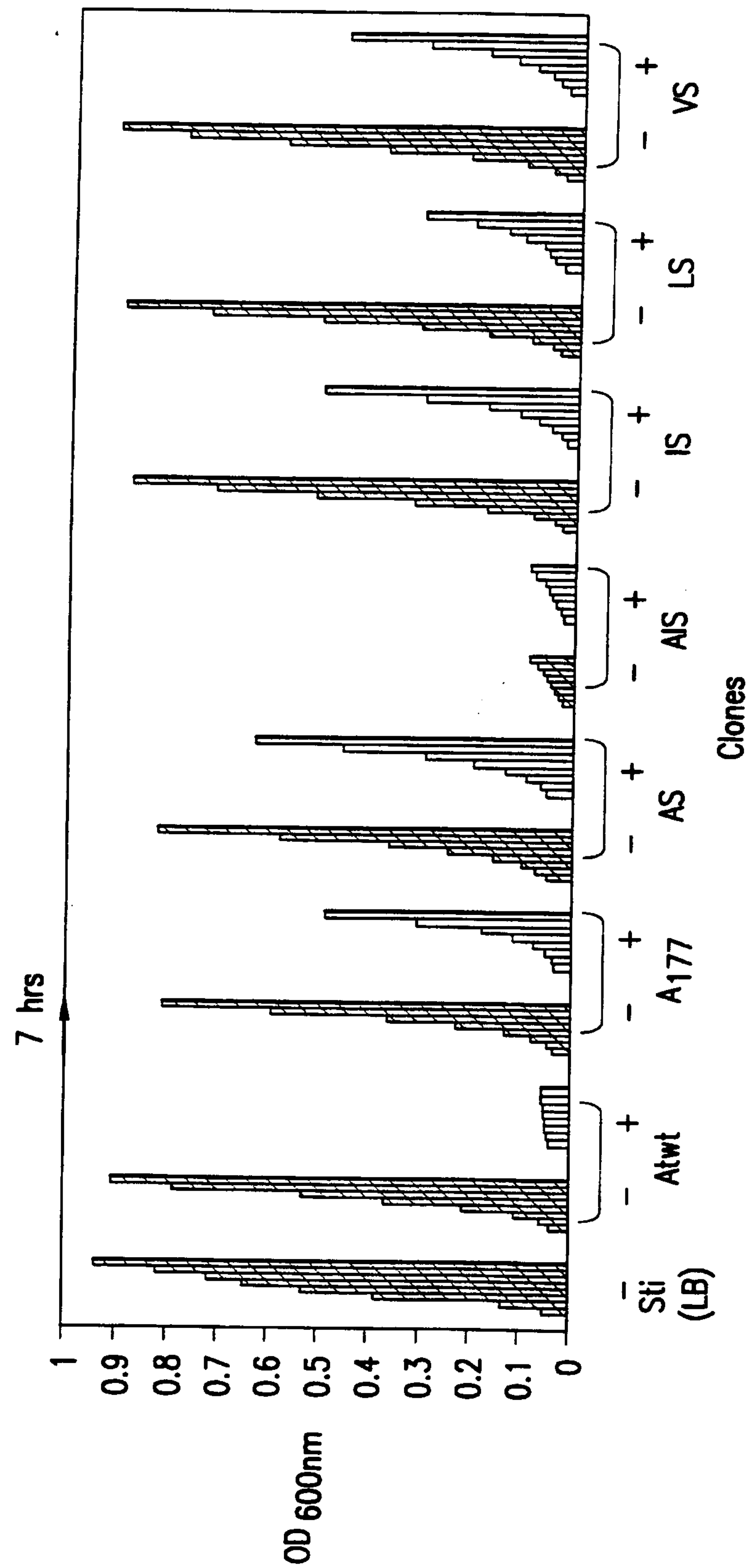
7 hrs
OD 600nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Sti (LB)
Atwt
A_{177}
AS
AIS
IS
LS
VS
Clones

FIG.6

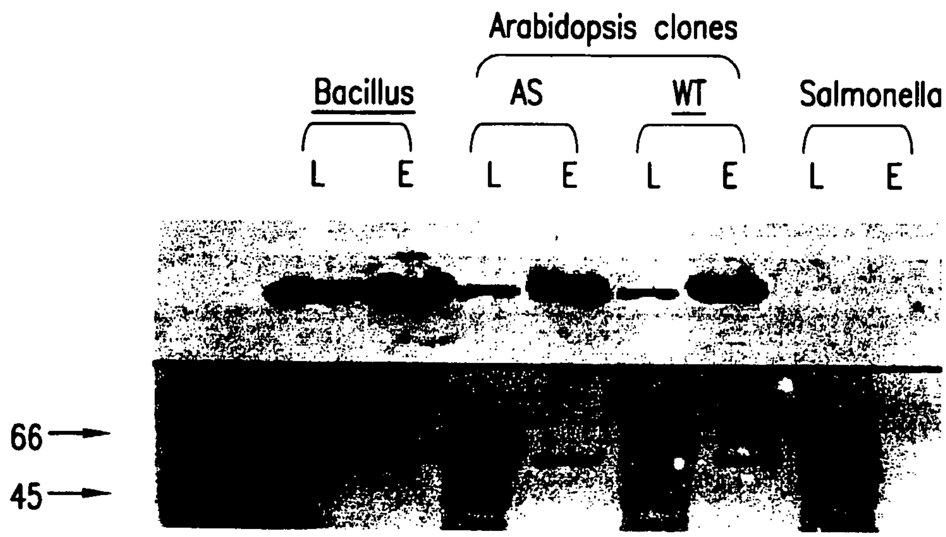
Arabidopsis clones
Bacillus
AS
WT
Salmonella
L E L E L E L E
66
45

FIG.7

SEQUENCE LISTING

<110> VALIGEN(US), INC.

<120> NON-TRANSGENIC HERBICIDE RESISTANT PLANTS

<130> 7991-086-228

<150> 60/158,027
<151> 1999-10-07

<150> 60/173,564
<151> 1999-12-30

<160> 44

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 2763
<212> DNA
<213> Arabidopsis thaliana

<400> 1
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gtgtcatact gccagtggtt atgataaatg ctcataccat accagagtca tagagttttt     180
ggttggtgaa agatttgacg gatgccttct tctcatttct caccaactcc ctccaaaccc     240
aacaaaatgt ttatattagc aaagccgcca aagtgtaaac gaaagtttat aaatttcatt     300
tctgtgatct tacgtaattg gaggaagatc aaaattttca atccccattc ttcgattgct     360
tcaattgaag tttctccgat ggcgcaagtt agcagaatct gcaatggtgt gcagaaccca     420
tctcttatct ccaatctctc gaaatccagt caacgcaaat ctcccttatc ggtttctctg     480
aagacgcagc agcatccacg agcttatccg atttcgtcgt cgtggggatt gaagaagagt     540
gggatgacgt taattggctc tgagcttcgt cctcttaagg tcatgtcttc tgtttccacg     600
gcggagaaag cgtcggagat tgtacttcaa cccattagag aaatctccgg tcttattaag     660
cttcctggct ccaagtctct atcaaatcgg atcctgcttc tcgctgctct gtctgaggta     720
tatatcactt cgtttcgtcc ttctctgtaa tctgaactta gattataaag attgatactt     780
taccattttg ctgtggtttt atagggaaca actgtagtgg acaacttgtt gaatagcgat     840
gacatcaatt acatgcttga tgcgttgaag agattgggac ttaatgtgga aactgacagt     900
gaaaataatc gtgctgtagt tgaaggatgt ggcgggatat tcccagcttc catagattca     960
aagagtgata tcgaacttta cctcggtaat gcaggaacag caatgcgtcc acttaccgct    1020
gcggtcactg ctgcaggtgg aaacgcaagg tagattgaag gagttgatgc ttcttggtat    1080
ttgatgttta aggaatggag cttttgttga tgctttatga tccatttatt ccagttatgt    1140
gcttgatggg gtgcctcgta tgagagaaag acctataggg gatttggttg ttggtcttaa    1200
gcagcttggt gctgatgttg aatgtactct tggaactaac tgccctcctg ttcgtgtcaa    1260
cgctaatggt ggccttcccg gtggaaaggt tagatcttgc aaatggcatg tgaatatgta    1320
atctcgttcc ttactctatg aacacttgca gaaatgtgtg ttcatcatag ccttagcttg    1380
acaagatttc agtttttaat ctactctcaa cggatggatc ctaaaataga atcggatttg    1440
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gagacatgtt atgcatttgc aggtgaagct ttctggatca attagtagtc agtacttgac    1560
tgctctgctc atgtctgctc ccttagctct tggagacgtc gagattgaga ttgtcgataa    1620
attaatttct gttccatatg ttgaaatgac attgaagttg atggaacgtt tcggggttag    1680
tgtcgagcat agtgatagct gggatcgttt ctttgtcaag ggcgggcaaa aatacaagta    1740
ggagttattc ttttcttcct tttctgaaat cacatccctt agcttgacaa tataatgact    1800
aaaaggtgaa tgattcaggt ctccgggtaa tgcgtatgta gaaggtgatg cttctagtgc    1860
atgttatttc ttggctggtg ctgccattac cggtgaaact gtcacagtcg aaggttgtgg    1920
aactaccagc ttgcaggtaa tatttgtaca ctgaatcatc gacgaggctg ttaagtttat    1980
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agtgaaattc gtctaggtca aagtttcatc ttttgacaag ttgtatataa catattcgca    2040
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ttgagaaaat gggatgtaaa gtgtcctgga cagagaacag tgtgactgtg acaggaccac    2160
ctagagatgc ttttggaatg agacacttgc gggctattga tgtcaacatg aacaaaatgc    2220
ctgatgtagc catgacccctt gccgtcgttg ctctctttgc tgacggtcca accaccatta    2280
gagatggtaa gtaaaaagct ctctcttata attaaggttt ctcaatattc atgatcactt    2340
aattctgttt ggttaatata gtggctagct ggagagtaaa ggagacagaa aggatgattg    2400
ccatttgcac agagcttaga aaagtaagag attcttatct ctctctttct gtctcttgac    2460
agtgctcatt ctaagtaatt agctcataaa tttgtgtgtt tgtgttcagc tgggagctac    2520
agtggaagaa ggttcagatt attgtgtgat aactccgccc aaaaaggtga aaacggcaga    2580
gattgataca tatgatgatc atagaatggc aatggcattc tctcttgcag cttgtgctga    2640
tgttccaatc accatcaacg actctggttg caccaggaaa accttccccg actacttcca    2700
agtacttgaa agaatcacaa agcactaaac aataaactct gtttttctt ctgatccaag    2760
ctt                                                                  2763
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      <210> 2
      <211> 520
      <212> PRT
      <213> Arabidopsis thaliana

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 1               5                  10                  15
Ile Ser Asn Leu Ser Lys Ser Ser Gln Arg Lys Ser Pro Leu Ser Val
            20                  25                  30
Ser Leu Lys Thr Gln Gln His Pro Arg Ala Tyr Pro Ile Ser Ser Ser
        35                  40                  45
Trp Gly Leu Lys Lys Ser Gly Met Thr Leu Ile Gly Ser Glu Leu Arg
    50                  55                  60
Pro Leu Lys Val Met Ser Ser Val Ser Thr Ala Glu Lys Ala Ser Glu
65                  70                  75                  80
Ile Val Leu Gln Pro Ile Arg Glu Ile Ser Gly Leu Ile Lys Leu Pro
                85                  90                  95
Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu Leu Ala Ala Leu Ser
            100                 105                 110
Glu Gly Thr Thr Val Val Asp Asn Leu Leu Asn Ser Asp Asp Ile Asn
        115                 120                 125
Tyr Met Leu Asp Ala Leu Lys Arg Leu Gly Leu Asn Val Glu Thr Asp
    130                 135                 140
Ser Glu Asn Asn Arg Ala Val Val Glu Gly Cys Gly Gly Ile Phe Pro
145                 150                 155                 160
Ala Ser Ile Asp Ser Lys Ser Asp Ile Glu Leu Tyr Leu Gly Asn Ala
                165                 170                 175
Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr Ala Ala Gly Gly
            180                 185                 190
Asn Ala Ser Tyr Val Leu Asp Gly Val Pro Arg Met Arg Glu Arg Pro
        195                 200                 205
Ile Gly Asp Leu Val Val Gly Leu Lys Gln Leu Gly Ala Asp Val Glu
    210                 215                 220
Cys Thr Leu Gly Thr Asn Cys Pro Pro Val Arg Val Asn Ala Asn Gly
225                 230                 235                 240
Gly Leu Pro Gly Gly Lys Val Lys Leu Ser Gly Ser Ile Ser Ser Gln
                245                 250                 255
Tyr Leu Thr Ala Leu Leu Met Ser Ala Pro Leu Ala Leu Gly Asp Val
            260                 265                 270
Glu Ile Glu Ile Val Asp Lys Leu Ile Ser Val Pro Tyr Val Glu Met
        275                 280                 285
```

```
Thr Leu Lys Leu Met Glu Arg Phe Gly Val Ser Val Glu His Ser Asp
    290                 295                 300
Ser Trp Asp Arg Phe Phe Val Lys Gly Gly Gln Lys Tyr Lys Ser Pro
305                 310                 315                 320
Gly Asn Ala Tyr Val Glu Gly Asp Ala Ser Ser Ala Cys Tyr Phe Leu
                325                 330                 335
Ala Gly Ala Ala Ile Thr Gly Glu Thr Val Thr Val Glu Gly Cys Gly
            340                 345                 350
Thr Thr Ser Leu Gln Gly Asp Val Lys Phe Ala Glu Val Leu Glu Lys
        355                 360                 365
Met Gly Cys Lys Val Ser Trp Thr Glu Asn Ser Val Thr Val Thr Gly
    370                 375                 380
Pro Pro Arg Asp Ala Phe Gly Met Arg His Leu Arg Ala Ile Asp Val
385                 390                 395                 400
Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu Ala Val Val Ala
                405                 410                 415
Leu Phe Ala Asp Gly Pro Thr Thr Ile Arg Asp Val Ala Ser Trp Arg
            420                 425                 430
Val Lys Glu Thr Glu Arg Met Ile Ala Ile Cys Thr Glu Leu Arg Lys
        435                 440                 445
Leu Gly Ala Thr Val Glu Glu Gly Ser Asp Tyr Cys Val Ile Thr Pro
    450                 455                 460
Pro Lys Lys Val Lys Thr Ala Glu Ile Asp Thr Tyr Asp Asp His Arg
465                 470                 475                 480
Met Ala Met Ala Phe Ser Leu Ala Ala Cys Ala Asp Val Pro Ile Thr
                485                 490                 495
Ile Asn Asp Ser Gly Cys Thr Arg Lys Thr Phe Pro Asp Tyr Phe Gln
            500                 505                 510
Val Leu Glu Arg Ile Thr Lys His
        515                 520
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<210> 3
<211> 33
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(33)

<400> 3

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Leu Gly Asn Ala Ala Thr Ala Met Arg Pro Leu
 1               5                  10
```

<210> 4
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<400> 4

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Leu Gly Asn Ala Ala Thr Ala Met Arg Pro Leu
 1               5                  10
```

<210> 5
<211> 33
<212> DNA

<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(33)

<400> 5
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ctc ggt aat gca gga ata gca atg cgt cca ctt                            33
Leu Gly Asn Ala Gly Ile Ala Met Arg Pro Leu
 1               5                  10
```

<210> 6
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<400> 6
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Leu Gly Asn Ala Gly Ile Ala Met Arg Pro Leu
 1               5                  10
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<210> 7
<211> 33
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(33)

<400> 7
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Leu Gly Asn Ala Ala Ile Ala Met Arg Pro Leu
 1               5                  10
```

<210> 8
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<400> 8
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Leu Gly Asn Ala Ala Ile Ala Met Arg Pro Leu
 1               5                  10
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<210> 9
<211> 33
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(33)

<400> 9
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Leu Gly Asn Ala Gly Ile Ala Met Arg Ser Leu
 1               5                  10
```

<210> 10
<211> 11
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<400> 10
Leu Gly Asn Ala Gly Ile Ala Met Arg Ser Leu
1 5 10

<210> 11
<211> 33
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<220>
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<222> (1)...(33)

<400> 11
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Leu Gly Asn Ala Ala Thr Ala Met Arg Ser Leu
1 5 10

<210> 12
<211> 11
<212> PRT
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<400> 12
Leu Gly Asn Ala Ala Thr Ala Met Arg Ser Leu
1 5 10

<210> 13
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<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(33)

<400> 13
ctc ggt aat gca gca ata gca atg cgt tca ctt 33
Leu Gly Asn Ala Ala Ile Ala Met Arg Ser Leu
1 5 10

<210> 14
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<400> 14
Leu Gly Asn Ala Ala Ile Ala Met Arg Ser Leu
1 5 10

<210> 15
<211> 33
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(33)

<400> 15
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Leu Gly Asn Ala Gly Val Ala Met Arg Ser Leu
 1               5                   10
```

<210> 16
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<400> 16
```
Leu Gly Asn Ala Gly Val Ala Met Arg Ser Leu
 1               5                  10
```

<210> 17
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<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(33)

<400> 17
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Leu Gly Asn Ala Gly Leu Ala Met Arg Ser Leu
 1               5                   10
```

<210> 18
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<400> 18
```
Leu Gly Asn Ala Gly Leu Ala Met Arg Ser Leu
 1               5                  10
```

<210> 19
<211> 33
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(33)

<400> 19

ctc ggt aat gca gca gta gca atg cgt cca ctt 33
Leu Gly Asn Ala Ala Val Ala Met Arg Pro Leu
1 5 10

<210> 20
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<400> 20
Leu Gly Asn Ala Ala Val Ala Met Arg Pro Leu
1 5 10

<210> 21
<211> 33
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(33)

<400> 21
ctc ggt aat gca gca tta gca atg cgt cca ctt 33
Leu Gly Asn Ala Ala Leu Ala Met Arg Pro Leu
1 5 10

<210> 22
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<400> 22
Leu Gly Asn Ala Ala Leu Ala Met Arg Pro Leu
1 5 10

<210> 23
<211> 3831
<212> DNA
<213> Brassisca napus

<220>
<221> modified_base
<222> 1...3831
<223> n=a, c, g, or t

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tctgaaatag ccgacgtgga taaagtactt aagacgtggc acattattat tggctactag 120
aaaaaaaact catacaccat cgtaggagtt ggggttggtg aagaatttga tgggtgcctc 180
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gacgacagtt ctatagaaaa gctttcaaat tcaatcaatg gcgcaatcta gcagaatctg 300
ccatggcgtg cagaacccat gtgttatcat ctccaatctc tccaaatcca accaaaacaa 360
atcacctttc tccgtctcct tgaagacgca tcagcctcga gcttcttcgt ggggattgaa 420
gaagagtgga acgatgctaa acggttctgt aattcgcccg gttaaggtaa cagcttctgt 480
ttccacgtcc gagaaagctt cagagattgt gcttcaacca atcagagaaa tctcgggtct 540

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cattaagcta cccggatcca aatctctctc caatcggatc ctccttcttg ccgctctatc    600
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aatttttcca aaatttttgg aggttatagg cttatgttac accattctag tctgcatctt    780
tcggtttgag actgaagaat tttatttttt aaaaaattat tatagggaac tactgtagtg    840
gacaacttgt tgaacagtga tgacatcaac tacatgcttg atgcgttgaa gaagctgggg    900
cttaacgtgg aacgtgacag tgtaaacaac cgtgcggttg ttgaaggatg cggtggaata    960
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<210> 24
<211> 1944

<212> DNA
<213> Petunia hybrida

<400> 24
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cttgtttttg gatctaaaaa actgaaaaat tcagcaaatt ctatgttggt tttgaaaaaa 180
gattcaattt ttatgcaaaa gttttgttcc tttaggattt cagcatcagt ggctacagca 240
cagaagcctt ctgagatagt gttgcaaccc attaaagaga tttcaggcac tgttaaattg 300
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agaattacta gtatgggcca gtgtaaggag tactattact ctttgcttat tttattgatt 1860
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tgtgtctaaa tgaaattgtt tgat 1944

<210> 25
<211> 1335
<212> DNA
<213> Zea mays

<400> 25
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acagtggttg ataacctgct gaacagtgag gatgtccact acatgctcgg ggccttgagg 180
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tacttgagtg ccttgctgat ggctgctcct ttggctcttg gggatgtgga gattgaaatc 600
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<210> 26
<211> 516
<212> PRT
<213> Brassisca napus

<400> 26
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Ile Ile Ser Asn Leu Ser Lys Ser Asn Gln Asn Lys Ser Pro Phe Ser
20 25 30
Val Ser Leu Lys Thr His Gln Pro Arg Ala Ser Ser Trp Gly Leu Lys
35 40 45
Lys Ser Gly Thr Met Leu Asn Gly Ser Val Ile Arg Pro Val Lys Val
50 55 60
Thr Ala Ser Val Ser Thr Ser Glu Lys Ala Ser Glu Ile Val Leu Gln
65 70 75 80
Pro Ile Arg Glu Ile Ser Gly Leu Ile Lys Leu Pro Gly Ser Lys Ser
85 90 95
Leu Ser Asn Arg Ile Leu Leu Leu Ala Ala Leu Ser Glu Gly Thr Thr
100 105 110
Val Val Asp Asn Leu Leu Asn Ser Asp Asp Ile Asn Tyr Met Leu Asp
115 120 125
Ala Leu Lys Lys Leu Gly Leu Asn Val Glu Arg Asp Ser Val Asn Asn
130 135 140
Arg Ala Val Val Glu Gly Cys Gly Gly Ile Phe Pro Ala Ser Leu Asp
145 150 155 160
Ser Lys Ser Asp Ile Glu Leu Tyr Leu Gly Asn Ala Gly Thr Ala Met
165 170 175
Arg Pro Leu Thr Ala Ala Val Thr Ala Ala Gly Gly Asn Ala Ser Tyr
180 185 190
Val Leu Asp Gly Val Pro Arg Met Arg Glu Arg Pro Ile Gly Asp Leu
195 200 205
Val Val Gly Leu Lys Gln Leu Gly Ala Asp Val Glu Cys Thr Leu Gly
210 215 220
Thr Asn Cys Pro Pro Val Arg Val Asn Ala Asn Gly Gly Leu Pro Gly
225 230 235 240
Gly Lys Val Lys Leu Ser Gly Ser Ile Ser Ser Gln Tyr Leu Thr Ala
245 250 255
Leu Leu Met Ala Ala Pro Leu Ala Leu Gly Asp Val Glu Ile Glu Ile
260 265 270
Ile Asp Lys Leu Ile Ser Val Pro Tyr Val Glu Met Thr Leu Lys Leu
275 280 285
Met Glu Arg Phe Gly Val Ser Ala Glu His Ser Asp Ser Trp Asp Arg
290 295 300
Phe Phe Val Lys Gly Gly Gln Lys Tyr Lys Ser Pro Gly Asn Ala Tyr
305 310 315 320
Val Glu Gly Asp Ala Ser Ser Ala Ser Tyr Phe Leu Ala Gly Ala Ala
325 330 335

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Ile Thr Gly Glu Thr Val Thr Val Glu Gly Cys Gly Thr Thr Ser Leu
            340                 345                 350
Gln Gly Asp Val Lys Phe Ala Glu Val Leu Glu Lys Met Gly Cys Lys
        355                 360                 365
Val Ser Trp Thr Glu Asn Ser Val Thr Val Thr Gly Pro Ser Arg Asp
    370                 375                 380
Ala Phe Gly Met Arg His Leu Arg Ala Val Asp Val Asn Met Asn Lys
385                 390                 395                 400
Met Pro Asp Val Ala Met Thr Leu Ala Val Val Ala Leu Phe Ala Asp
                405                 410                 415
Gly Pro Thr Thr Ile Arg Asp Val Ala Ser Trp Arg Val Lys Glu Thr
            420                 425                 430
Glu Arg Met Ile Ala Ile Cys Thr Glu Leu Arg Lys Leu Gly Ala Thr
        435                 440                 445
Val Glu Glu Gly Ser Asp Tyr Cys Val Ile Thr Pro Pro Ala Lys Val
    450                 455                 460
Lys Pro Ala Glu Ile Asp Thr Tyr Asp Asp His Arg Met Ala Met Ala
465                 470                 475                 480
Phe Ser Leu Ala Ala Cys Ala Asp Val Pro Val Thr Ile Lys Asp Pro
                485                 490                 495
Gly Cys Thr Arg Lys Thr Phe Pro Asp Tyr Phe Gln Val Leu Glu Ser
            500                 505                 510
Ile Thr Lys His
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      <210> 27
      <211> 516
      <212> PRT
      <213> Petunia hybrida

      <400> 27
Met Ala Gln Ile Asn Asn Met Ala Gln Gly Ile Gln Thr Leu Asn Pro
 1               5                  10                  15
Asn Ser Asn Phe His Lys Pro Gln Val Pro Lys Ser Ser Ser Phe Leu
            20                  25                  30
Val Phe Gly Ser Lys Lys Leu Lys Asn Ser Ala Asn Ser Met Leu Val
        35                  40                  45
Leu Lys Lys Asp Ser Ile Phe Met Gln Lys Phe Cys Ser Phe Arg Ile
    50                  55                  60
Ser Ala Ser Val Ala Thr Ala Gln Lys Pro Ser Glu Ile Val Leu Gln
65                  70                  75                  80
Pro Ile Lys Glu Ile Ser Gly Thr Val Lys Leu Pro Gly Ser Lys Ser
                85                  90                  95
Leu Ser Asn Arg Ile Leu Leu Leu Ala Ala Leu Ser Glu Gly Thr Thr
            100                 105                 110
Val Val Asp Asn Leu Leu Ser Ser Asp Asp Ile His Tyr Met Leu Gly
        115                 120                 125
Ala Leu Lys Thr Leu Gly Leu His Val Glu Glu Asp Ser Ala Asn Gln
    130                 135                 140
Arg Ala Val Val Glu Gly Cys Gly Gly Leu Phe Pro Val Gly Lys Glu
145                 150                 155                 160
Ser Lys Glu Glu Ile Gln Leu Phe Leu Gly Asn Ala Gly Thr Ala Met
                165                 170                 175
Arg Pro Leu Thr Ala Ala Val Thr Val Ala Gly Gly Asn Ser Arg Tyr
            180                 185                 190
Val Leu Asp Gly Val Pro Arg Met Arg Glu Arg Pro Ile Ser Asp Leu
        195                 200                 205
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Val Asp Gly Leu Lys Gln Leu Gly Ala Glu Val Asp Cys Phe Leu Gly
    210                 215                 220
Thr Lys Cys Pro Pro Val Arg Ile Val Ser Lys Gly Gly Leu Pro Gly
225                 230                 235                 240
Gly Lys Val Lys Leu Ser Gly Ser Ile Ser Ser Gln Tyr Leu Thr Ala
                245                 250                 255
Leu Leu Met Ala Ala Pro Leu Ala Leu Gly Asp Val Glu Ile Glu Ile
            260                 265                 270
Ile Asp Lys Leu Ile Ser Val Pro Tyr Val Glu Met Thr Leu Lys Leu
        275                 280                 285
Met Glu Arg Phe Gly Ile Ser Val Glu His Ser Ser Ser Trp Asp Arg
    290                 295                 300
Phe Phe Val Arg Gly Gly Gln Lys Tyr Lys Ser Pro Gly Lys Ala Phe
305                 310                 315                 320
Val Glu Gly Asp Ala Ser Ser Ala Ser Tyr Phe Leu Ala Gly Ala Ala
                325                 330                 335
Val Thr Gly Gly Thr Ile Thr Val Glu Gly Cys Gly Thr Asn Ser Leu
            340                 345                 350
Gln Gly Asp Val Lys Phe Ala Glu Val Leu Glu Lys Met Gly Ala Glu
        355                 360                 365
Val Thr Trp Thr Glu Asn Ser Val Thr Val Lys Gly Pro Pro Arg Ser
    370                 375                 380
Ser Ser Gly Arg Lys His Leu Arg Ala Ile Asp Val Asn Met Asn Lys
385                 390                 395                 400
Met Pro Asp Val Ala Met Thr Leu Ala Val Val Ala Leu Tyr Ala Asp
                405                 410                 415
Gly Pro Thr Ala Ile Arg Asp Val Ala Ser Trp Arg Val Lys Glu Thr
            420                 425                 430
Glu Arg Met Ile Ala Ile Cys Thr Glu Leu Arg Lys Leu Gly Ala Thr
        435                 440                 445
Val Glu Glu Gly Pro Asp Tyr Cys Ile Ile Thr Pro Pro Glu Lys Leu
    450                 455                 460
Asn Val Thr Asp Ile Asp Thr Tyr Asp Asp His Arg Met Ala Met Ala
465                 470                 475                 480
Phe Ser Leu Ala Ala Cys Ala Asp Val Pro Val Thr Ile Asn Asp Pro
                485                 490                 495
Gly Cys Thr Arg Lys Thr Phe Pro Asn Tyr Phe Asp Val Leu Gln Gln
            500                 505                 510
Tyr Ser Lys His
        515

      <210> 28
      <211> 444
      <212> PRT
      <213> Zea mays

      <400> 28
Ala Gly Ala Glu Glu Ile Val Leu Gln Pro Ile Lys Glu Ile Ser Gly
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Thr Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu
            20                  25                  30
Leu Ala Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu Leu Asn
        35                  40                  45
Ser Glu Asp Val His Tyr Met Leu Gly Ala Leu Arg Thr Leu Gly Leu
    50                  55                  60
Ser Val Glu Ala Asp Lys Ala Ala Lys Arg Ala Val Val Val Gly Cys
65                  70                  75                  80
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Gly Gly Lys Phe Pro Val Glu Asp Ala Lys Glu Glu Val Gln Leu Phe
85 90 95
Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr
100 105 110
Ala Ala Gly Gly Asn Ala Thr Tyr Val Leu Asp Gly Val Pro Arg Met
115 120 125
Arg Glu Arg Pro Ile Gly Asp Leu Val Val Gly Leu Lys Gln Leu Gly
130 135 140
Ala Asp Val Asp Cys Phe Leu Gly Thr Asp Cys Pro Pro Val Arg Val
145 150 155 160
Asn Gly Ile Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser Gly Ser
165 170 175
Ile Ser Ser Gln Tyr Leu Ser Ala Leu Leu Met Ala Ala Pro Leu Ala
180 185 190
Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser Ile Pro
195 200 205
Tyr Val Glu Met Thr Leu Arg Leu Met Glu Arg Phe Gly Val Lys Ala
210 215 220
Glu His Ser Asp Ser Trp Asp Arg Phe Tyr Ile Lys Gly Gly Gln Lys
225 230 235 240
Tyr Lys Ser Pro Lys Asn Ala Tyr Val Glu Gly Asp Ala Ser Ser Ala
245 250 255
Ser Tyr Phe Leu Ala Gly Ala Ala Ile Thr Gly Gly Thr Val Thr Val
260 265 270
Glu Gly Cys Gly Thr Thr Ser Leu Gln Gly Asp Val Lys Phe Ala Glu
275 280 285
Val Leu Glu Met Met Gly Ala Lys Val Thr Trp Thr Glu Thr Ser Val
290 295 300
Thr Val Thr Gly Pro Pro Arg Glu Pro Phe Gly Arg Lys His Leu Lys
305 310 315 320
Ala Ile Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu
325 330 335
Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Ala Ile Arg Asp Val
340 345 350
Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Val Ala Ile Arg Thr
355 360 365
Glu Leu Thr Lys Leu Gly Ala Ser Val Glu Glu Gly Pro Asp Tyr Cys
370 375 380
Ile Ile Thr Pro Pro Glu Lys Leu Asn Val Thr Ala Ile Asp Thr Tyr
385 390 395 400
Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys Ala Glu
405 410 415
Val Pro Val Thr Ile Arg Asp Pro Gly Cys Thr Arg Lys Thr Phe Pro
420 425 430
Asp Tyr Phe Asp Val Leu Ser Thr Phe Val Lys Asn
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<210> 29
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 29
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cgag 64

<210> 30
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 30
cgtttccacc tgcagcagtg accgcagcgg taagtggacg cattgctatt gctgcattac 60
cgag 64

<210> 31
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 31
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cgag 64

<210> 32
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 32
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cgag 64

<210> 33
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 33
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cgag 64

<210> 34
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 34
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cgag 64

<210> 35
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 35
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cgag 64

<210> 36
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 36
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<210> 37
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 37
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cgag 64

<210> 38
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutant primer

<400> 38
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cgag 64

<210> 39
<211> 5
<212> PRT
<213> Petunia hybrida

<400> 39
Leu Phe Leu Gly Asn
1 5

<210> 40
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 40
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<210> 41
<211> 41
<212> DNA
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<220>
<223> Primer

<400> 41
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<210> 42
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 42
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<210> 43
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<220>
<223> Primer

<400> 43
gcggatcctc aggatttttt cgaaagctta tttaaatg 38

<210> 44
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<212> DNA
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<220>
<223> Primer

<400> 44
gaaagcgtcg gagattgtac 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/27941

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01H 1/06; C07H 21/04; C12N 5/04, 9/00, 15/01, 15/09, 15/29, 15/87
US CL : 435/183, 410, 413, 418; 530/370; 536/23.1, 23.2; 800/276, 278, 300,300.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/183, 410, 413, 418; 530/370; 536/23.1, 23.2; 800/276, 278, 300,300.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P --- Y	US 6,066,786 ROSE-FRICKER) 23 May 2000 (23.05.00), entire reference.	1-4, 9 --- 5-8, 10-13
X --- Y	GORLANI et al. A glyphosate-resistant 5-enol-pyruvyl-shikimate-3-phosphate synthase confers tolerance to a maize cell line. Plant science 1992, Vol. 85, pages 9-15, entire reference.	1-4, 9 --- 5-8, 10-13
Y	PADGETTE et al. Site-directed mutagenesis of a conserved region of the 5-enolpyruvylshikimate-3-phosphate synthase active site. J. biol. chem. 25 November 1991, Vol. 266, No. 33, pages 22364-22369, especially Table 1, page 22365.	14--23
Y	US 5,756,325 A (KMIEC) 26 May 1998 (26.05.98), entire reference.	14-23
Y,P	US 6,004,804 A (KUMAR et al) 21 December 1999 (21.12.99), entire reference.	14-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 21 November 2000 (21.11.2000)	Date of mailing of the international search report JAN 19 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Dorothea Lawrence For David Kruse Telephone No. 703-308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/27941

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☒ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-23, drawn to a non-transgenic herbicide resistant plant that expresses a mutant EPSPS gene product and a method of producing said plant.

Group II, claim(s) 24, drawn to an isolated mutant EPSPS protein containing amino acid substitutions at specific sequence sites.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The isolated mutant EPSPS protein of Group II lacks a corresponding technical feature with the transgenic-herbicide resistant plant and the method of producing said plant of Group I. The isolated mutant EPSPS protein of Group II requires multiple amino acid substitution and the EPSPS expressed in the transgenic plant of Group I only requires one amino acid substitution.

Continuation of B. FIELDS SEARCHED Item 3:
(1) EAST (USPAT, Derwent, JPO, EPO); natural glyphosate resistance, non-transgenic herbicide reistance, recombinagenic oligonucleobase, site directed mutation [in] plant(s).
(2) STN (BIOSIS, AGRICOLA, EMBASE, CAPLUS); EPSPS Mutant/Mutation [in] plant(s), Natural glyphosate resistance.
(3) Sequence Search of SEQ ID NO:2